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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

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BIOLOGICAL BULLETIN.

NOTES ON THE REGENERATION OF GONIONEMA.

G. T. HARGITT.

The experiments of which this paper presents a résumé were conducted at the Marine Biological Laboratory during the summer of 1901. The purpose in view was such a review of previous work done by C. W. Hargitt '97,[1] '99,[2] and by Morgan '99,[3] as would confirm or correct, or possibly extend, the conclusions then announced.

The work was suggested by and the experiments carried on under the direction of C. W. Hargitt, whose advice and oversight has been largely instrumental in making possible the preparation of this paper.

By way of introduction it may be stated that three general series of experiments were projected:

I. Experiments upon the regeneration of the marginal organs of the medusæ.

II. Experiments on the regeneration of the manubrium.

III. Experiments upon the regeneration of the radial canals.

Under each of these series several sets of experiments were conducted, and under each set a sufficient number of specimens operated upon to insure reasonable precautions against failure or unwarranted conclusions from limited observations. In other words the repeated experiments operated as controls or checks against exceptional results in either direction.

In general the methods followed were similar to those of previous experiments. The medusæ were operated upon very soon after being brought into the laboratory, being cut into desired forms by means of clean scissors as the animals were held gently, by means of delicate forceps, in dishes of perfectly fresh sea water. After the operation the specimens were immediately transferred to clean sea water, which was afterward changed at least daily and sometimes oftener. The specimens were fed from time to time with bits of freshly killed shrimp or small fish. The specimens usually seemed to thrive quite well considering the artificial conditions under which they were placed and the mutilation to which the operations had subjected them. Occasionally, as will be noted later, whole sets under a given experiment would seem to decline or go bad, a fact doubtless due to accidental contamination of the water in which they were kept.

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[1] *Zoölogical Bulletin*, Vol. I., p. 27.

[2] BIOLOGICAL BULLETIN, Vol. I., p. 35.

[3] *The American Naturalist*, Vol. XXXIII., p. 939.

I. Experiments upon the Regeneration of the Marginal Organs of the Medusæ.

Hargitt '97, and Morgan 99, found that when the entire margin was removed new tentacles regenerated though in their investigations they remained rudimentary and bud-like. I repeated experiments of a similar kind to see in how far my results would confirm theirs. Five sets of experiments were tried.

In all cases the cut edges contracted more or less. In the first set the contraction continued till the edges had nearly met and the bell was spherical and had only a very small opening into the subumbrella. Five days after the operation small refractile bulbs were found at the lower distal pole of the sphere, in several specimens. The next day tentacles had begun to grow from these bulbs. On the ninth day two to four tentacles were found on those in which the bulbs had earlier formed. These tentacles were of considerable length, had the rings of nematocysts and the suctorial pads found in the mature tentacles (Fig. 1). As the opening was so small as to prevent the taking of food, the specimens died before further developments could take place. Particular care was taken to remove the entire margin with all the tentacles and bulbs at the time of operation, so that these regenerated tentacles were undoubtedly of new growth.

In the second set the cut edges contracted, though not into a sphere, and a large opening into the subumbrella was left. No velum formed in any of the specimens. In two days several bulbs had formed on some of the margins. In four days marginal canals and several bulbs had formed in other specimens. Quite a large number of bulbs were present; in some cases thirty were counted, while in others as many as forty were found. These bulbs are probably an aggregation of new tissue and do not represent the bulbs usually found at the bases of mature tentacles, as these latter normally form after the tentacles have been regenerated. In Fig. 2 the marginal canal and bulbs are shown. From several of the bulbs elongations resembling young tentacles had formed. Specimens were kept two weeks after the bulbs were first found and though the bulbs increased from two or three to twenty or forty the tentacles did not develop beyond what is shown in Fig. 2.

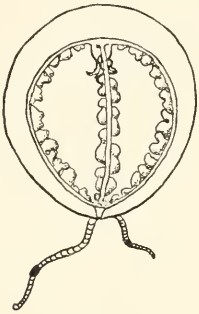

FIG. I.

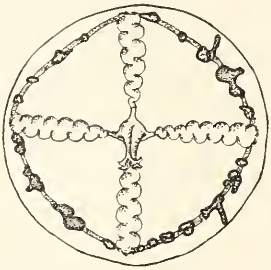

FIG. 2.

In the third set the margins contracted somewhat, but on the second day all evaginated and in some cases the bell began to degenerate. One of the specimens turned right side out and twelve hours later evaginated again and then gradually disintegrated. All the specimens died before any regeneration took place. In the fourth set all died within a week without having regenerated. One specimen flattened out and attached itself to the bottom of the dish by the manubrium. Several of them evaginated and in all the bell slowly disintegrated and the medusæ soon died.

The fact that in these two sets nearly all evaginated and all died within a week, and that this happened only in this experiment, needs some further explanation. The same care and methods were used in this experiment as in all others, with the exception that in the third set the water was taken from the tap in the laboratory, while in most of the others it was taken directly from the open harbor. Since in these two sets the medusæ were placed in smaller dishes, the consequent overcrowding may have been the cause of their death, through lack of aëration.

In order to ascertain whether the regeneration of new tentacles would take place more rapidly if a portion of the margin was left, than when it was entirely removed, three quarters of the margin was excised leaving one quarter of the original number of tentacles still present. The part from which the tentacles had been removed gradually contracted till it occupied only about one quarter or one half of the new margin. Thus two or three canals were crowded into less than half of the margin and two of them sometimes fused as shown in Fig. 3. New marginal canals were fully formed in about a week, and the circulation of the fluid in them could be observed. In ten or twelve days small bulbs were found on the margins of some, and from these bulbs tentacles formed, while at about the same time new otocysts developed. As suggested under the second set these bulbs are probably aggregations of new tissue, and are perhaps comparable to the tentacle "anlagen" in regenerating hydroids. Fig. 4 shows the portion of the margin from which the tentacles were excised, *o* represents otocysts and *t* the new tentacles. It will be seen that the pads or bulbs *b* found at the bases of the old tentacles are lacking in the new tentacles at this stage, and form only after the new tentacles have attained considerable growth. This fact has been previously observed and noted by Hargitt[1] 1901. The tentacles

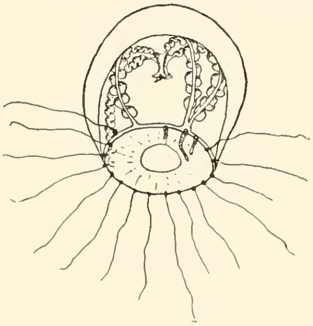

FIG. 3.

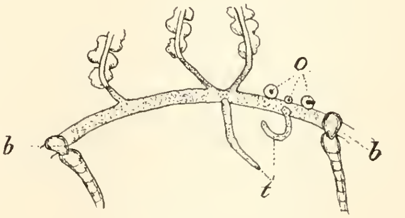

FIG. 4.

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[1] BIOLOGICAL BULLETIN, Vol. II., p. 244.

grew slowly and in two or three weeks some specimens had developed only two to four tentacles, though several small bulbs were present. In other specimens more tentacles formed, Fig. 5 showing seventeen bulbs and small tentacles on the regenerated margin.

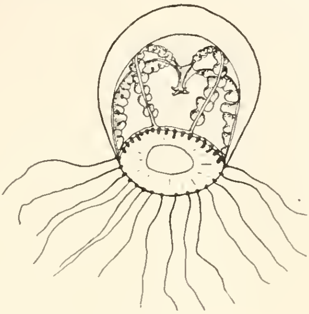

FIG. 5.

II. EXPERIMENTS ON THE REGENERATION OF THE MANUBRIUM.

In some cases the manubrium was removed by a circular cut passing entirely through the jelly of the bell. The size of the opening left varied in the different specimens but was not very large in any individual. In other cases the upper half of the bell was removed, thus leaving a very large opening. The rate of healing did not seem to be at all dependent upon the size of the opening, for, as noted below, some of those in which the upper half of the bell was removed took less time to heal than some in which only the manubrium was cut out. In all cases the cut edges gradually contracted till they were touching. As soon as they met they began to fuse and in a very short time the wound was entirely healed. The rate of contraction and healing was not the same in all the specimens.

In the first set the contraction was slow, the cut did not completely heal until 60 hours after the operation. In the second set the contraction was quite rapid. Twelve hours after the operation the cut edges had met and twelve hours later had entirely healed in half the specimens, though in the rest of the specimens of this set 60–70 hours were required. In the third set the cut edges had met and fused at the end of 48 hours. In the fourth set, although the upper half of the bell was removed, the edges contracted quite rapidly and in 36–48 hours had fused. In this last set the typical bell shape of the medusa could not be assumed because of the large amount of the bell removed. In contracting the margin was drawn up and when the edges had fused the medusa was flattened and the bell very shallow. Sometimes the center of the bell was depressed (Fig. 6), though this disappeared later. Occasionally the bell was twisted somewhat, the margin turned under and a tentacle thrust through the hole in the top of the bell. This of course delayed the healing of the wound. In the four sets of this experiment, after the cut edges had fused no scar or sign of the fusion was present.

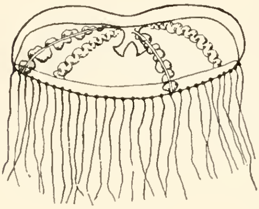

FIG. 6.

By the time the wound in the bell had closed, or very soon after, the free ends of the radial canals had also met and fused. The manubrium developed slowly. In 48–80 hours after the canals had fused a gastric pouch had usually formed. This is simply a large pouch into which the canals lead, and at first has no opening to the exterior. Fig. 7, *a*, shows the gastric pouch before the development of the manubrium. From these pouches the manubria develop. In some cases they had completely formed in four days after the operation. In other cases a week or ten days was necessary for their complete development. Quite common was the formation of double or bifurcated manubria. In the four sets of this experiment 21 per cent. developed double manubria. The formation of the double manubrium was followed out in detail in one specimen. Fig. 7, *a*, shows the large gastric pouch as it first appeared, with no mouth opening. A small diverticulum from this pouch was at first taken for the beginning of a new radial canal, but it developed into another pouch as shown in Fig. 7, *b*. From each of these pouches a

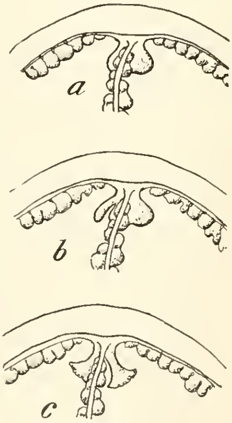

FIG. 7.

manubrium developed as shown in Fig. 7, *c*. All stages were found, from those in which each manubrium was distinct and had a separate pouch, through those in which two manubria were attached to a single pouch, and finally to those in which a single manubrium was branched or bifurcated at its distal end.

The fact that such a considerable per cent. developed double manubria assumes new interest when it is noted that this is not simply the result of artificial mutilation and regeneration. Several specimens of *Gonionema* have been taken from the eel pond near the laboratory, in which double or bifurcated manubria were present. Furthermore, this is not limited to *Gonionema*. During the summer of 1902 a specimen of *Oceania languida* was taken by C. W. Hargitt, in the "tow" in Vineyard Sound, in which two manubria were present. In this specimen three radial canals met normally in the center of the subumbrella. The fourth canal was not complete, extending from the marginal canal only about half way to the center. At the union of the three canals a large four-lobed manubrium was present. At the inner end of the fourth short canal was a small manubrium. This small manubrium was only three-lobed and had three oral frills, and in this resembled some of the regenerated manubria in *Gonionema* which had only two or three lobes. This small manubrium in *Oceania* may perhaps be interpreted as a case of adaptive regeneration. The short canal not having any direct connection with the center of the chymiferous system may have been nearly, if not entirely, deprived of the circulation of the chymiferous fluid, and thus threatened with atrophy ; and the new manubrium may have been formed to remedy this condition. This would be more important inasmuch as the gonad on this canal was as perfectly developed as those on the other canals. In both the cases just mentioned both of the manubria were active and functional.

In the second set the wound entirely healed as noted above, and then in two specimens the bell evaginated. The next day the evaginated ones died without having developed manubria or gastric pouches. In one specimen, Fig. 8, the canals did not unite as in former cases but in the form of a ring, and two manubria formed on opposite sides of the ring. They did not form at the same time, one being formed a day or two earlier than the other. The presence of these rings is not uncommon in specimens otherwise normal, as noted by Hargitt, 1901. As has already been noted, in the fourth set the upper half of the bell was removed. When the wound had healed the bell was flattened and elongated. It was therefore impossible for the canals to unite in the usual way. The two canals at the ends of the elongated bell united and between these two pairs of canals a straight connecting canal then regenerated, thus completing the chymiferous system. The manubrium then regenerated from these straight connecting canals, but not till the canal was completely formed and functional. If the manubrium formed at the union of the connecting canal with one of the pairs, it was triangular in shape and three-lobed as in Fig. 9. If it formed in the center of the connecting canal it was two- or four-lobed.

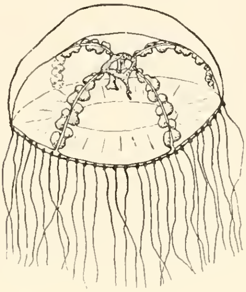

Fig. 8.

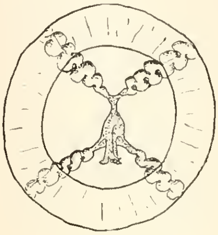

Fig. 9.

III. Experiments Upon the Regeneration of the Radial Canals.

The medusæ were first cut in a vertical interradial plane. Thus each piece had two canals and half the tentacles. The cut sometimes passed through the manubrium and sometimes to one side. Thus some pieces would have a part of the manubrium, others the entire manubrium and in the rest the manubrium would be entirely lacking. The cut edges gradually approached each other and finally met and fused, and the typical medusa-form was thus assumed. Occasionally the manubrium projected between the cut edges and delayed the healing. Usually, however, the fusion was complete in two to four days. During the process of healing the cut edge of the margin usually drew up somewhat, so that when the fusion was complete there was a scallop or notch in the margin of the bell. The ends of the marginal canal fused and a new radial canal formed along the line of union of the cut edges of the bell. This new canal elongated and the notch in the margin gradually disappeared. Morgan in describing a similar experiment says, "Along the line where the cut edges fused together a scar is present that resembles somewhat a third canal, but the third canal did not develop." While in his experiments this may have been the case, my own show conclusively the development of a third canal. Hargitt, '99, refers to the formation of new canals though he did not demonstrate their functional activity. Along the line of fusion of the cut edges a deposition of pigment is present which might be mistaken for a scar unless carefully examined. At first it is perhaps not a functional canal, but two or three days after the fusion the movement of the chymiferous fluid in the new canal was plainly observed, not only by myself but by others to whom it was pointed out. Furthermore in the closing of the bell, when the oral half had been removed, no sign of a scar or pigmentation was found. Such a scar or pigmented line, therefore, is not simply the sign of the fusion of cut edges, but represent the regeneration of a new organ along what is probably the line of least resistance.

This experiment was varied by cutting out one quadrant with its radial canal, leaving three canals, three quarters of the number of tentacles and the manubrium or a part of the manubrium. The fusion of the cut sides took place in about the same time as in the preceding experiment, though the incidental protrusion of the manubrium through the opening and the consequent delay in healing was more marked. On the fourth day the fusion was complete and a new canal had formed. Fig. 10 shows the new canal at *a*, the scallop in the margin is also shown. The latter gradually disappeared as in the former experiment and the bell margin became quite symmetrical.

The development of gonads on the new canal did not take place while the specimens were under observation. This would undoubtedly occur somewhat slowly if at all. This, however, is not a constant feature in normal specimens, in many of which gonads are lacking on one or more canals. Occasionally the fusion of the cut edges and the formation of new canals resulted in apparent abnormalities. Fig. 11 shows such a case in which the new canal *a* has anastomosed with one of the old ones. But this again has its counterpart among normal specimens in a state of nature.

The single quadrants which were cut out in the preceding experiment were placed in separate dishes. These contained a single canal, in some cases a part of a manubrium. The contraction of the cut edges took place as usual and in from three to four days the typical medusa-form had been assumed, the edges of the margin and marginal canal had fused as had also the velum. The apex of the quadrant was flexed toward the margin and when the fusion was complete this portion, and with it the proximal end of the canal, was joined to or very near, the marginal canal. In specimens having a portion of the old manubrium this was also carried downward and fused with the marginal canal and a gastric pouch formed at the point of union, and when the new manubrium was finally regenerated it protruded beyond the edge of the velum in the same horizontal plane. In specimens where no portion of the old manubrium was present usually entirely new ones regenerated but in the same general position as in the former. These new medusæ were of course only about one fourth the size of the original specimen and with only a proportional number of tentacles.

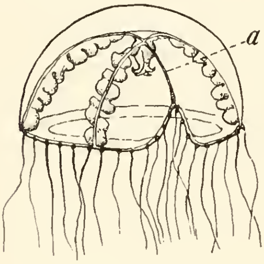

Fig. 10.

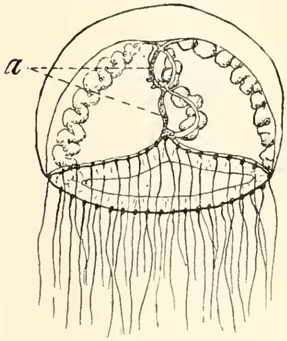

Fig. 11.

Summary and Review.

Hargitt, '97, showed for the first time that a medusa when divided into two or several pieces had the capacity to recover the original form, and that various organs, as manubria, canals and tentacles, were also regenerated. He also showed that the process was a gradual and orderly one. Further experiments in '99 by the same author showed that at least in case of radial canals and manubria the process was one of actual generation of new tissue and not merely the recasting of parts of old tissue into new forms.

Morgan, '99, experimenting on the same medusæ, confirmed these results to a certain extent, but claimed that new canals were not regenerated. He showed, moreover, that when cut into quadrants or smaller portions while a similar recovery of form was common, the resulting medusa was not typical in that in many cases there were lacking certains organs, as the typical number of canals, gonads, etc. And furthermore in case of smaller pieces where new manubria were regenerated it was not in the normal position. This observer also claimed that where only a portion of the margin was removed the regeneration of tentacles was more prompt and better developed than where the emargination had been complete.

As will have been seen, my own experiments confirm in part those of both these observers, and in part extend their results, by showing conclusively the regeneration and *functional activity* of both radial and marginal canals. My experiments show that when the division of the medusa was in vertical interradial planes that the resulting half medusæ were almost always perfectly symmetrical, while Morgan claimed they were otherwise, in that the manubrium was eccentrically located. This, so far as my experiments go, was only true when the section itself was quite unequal.

I was also able to obtain better results in regeneration of tentacles than either of the authors named, as an inspection of several of the figures will show. Concerning the experiments upon small pieces of medusæ, and regeneration of tentacles of partially emarginated specimens my own confirmed in almost all details those of Morgan.

As to the *processes* involved in these phases of regeneration my experiments confirm in the main those of both Hargitt and Morgan.

Hargitt, '97, says concerning it, "there seems to be an intrinsic potency to recast itself into the morphological equivalent of the original." Morgan says the process "is one of rounding up of the piece in direction of least resistance. The meeting of the edges may sometimes be due to simple accidental meeting of the bent-in portions." My own experiments showed no evidence of a merely accidental or mechanical process, but rather self-directive activity inherent in each portion of the individual. The process was in all cases gradual and orderly and the meeting of the edges too exact and certain to be explained as the result of mere accident.

SYRACUSE UNIVERSITY, THE ZOÖLOGICAL LABORATORY,
September 10, 1902.

NOTES ON A FEW MEDUSÆ NEW TO WOODS HOLL.

CHAS. W. HARGITT.

The following notes on a few medusæ taken during the current summer in the region of Woods Holl may serve as a preliminary account of work under way which will present a synopsis of the medusoid fauna of the region and which it is hoped may be ready within the year.

During the summer several expeditions for collecting were made to localities within fifty to seventy-five miles, most of which were made within a single day on board the launch "Phalarope," of the United States Fish Commission Laboratory, under direction of Dr. H. M. Smith, to whose courtesy I am under obligations, as also to Mr. Vinal N. Edwards for many favors.

The first excursion, and the one most prolific in specimens, was made to the region of the Gulf Stream, known as the Tile-fishing Station, latitude 40° 10′ 45″, longitute 70° 20′ 30″, by the schooner "Grampus," Captain Hahn, July 30 to August 2.

The material was taken by means of the tow-net, mostly from the surface, though in a few cases hauls were made from depths of from fifteen to twenty-five fathoms. Further reference to results of these deeper hauls will be made in another connection.

The medusæ taken include representatives of at least twelve genera and probably eighteen species. Several species of siphonophores are as yet undetermined. Specimens of *Oceania languida* and of *Epenthesis foliata* were very abundant, but these were taken most plentifully near Gay Head and No Mans Land. The following named species have been identified and of those not formerly recorded sketches and brief descriptions are given.

Oceania languida, taken in great abundance chiefly from a depth of about fifteen fathoms in water of seventeen fathoms. They were taken in the surface tow but in fewer numbers.

Epenthesis foliata, likewise taken in considerable numbers at similar ranges of depth.

Very few specimens of *Bougainvilla superciliaris* partly from the surface and partly from a lower depth.

Nemopsis bachei, about twenty specimens taken from deeper towing in Vineyard Sound.

Lafœa calcarata taken in surface tow near Gulf Stream. This is more usually a littoral form and its presence at this distance from land is not common. It is fairly common in the surface tow at Woods Holl.

Obelia were found in considerable numbers at the surface and near shore. Several species were taken, two of which, *O. geniculata* and *O. flabellata* were most common.

Aglaura hemistoma, numerous specimens taken at surface near the Gulf Stream. So far as I am aware this is the first record of this medusa within this region. The following cut, Fig. 1, will give a good general impression of this beautiful medusa. In size the specimens varied from two to four mm. in height by about half that in width. It was not possible to distinguish any sense organs on the specimens, the preservation in formalin having apparently rendered the otocysts, if present, indistinguishable. Incidentally it may be mentioned that this is a familiar effect with specimens preserved in formalin of any considerable strength. Even in 5 per cent. solutions it has this effect in many cases. The oral end of the manubrium is reddish in color, the gonads, which are borne just above the gastric pouch, are yellowish-white or brownish. The peduncle of the manubrium is long and gelatinous. In the specimens the tentacles had all been apparently broken off close to the margin so that only the short bases were remaining. Radial canals are eight in number and extend downward upon the peduncle.

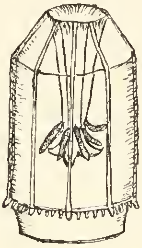

FIG. 1.

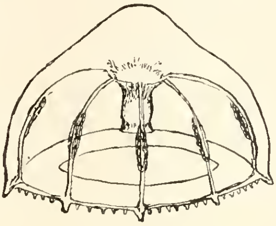

FIG. 2.

Several specimens of an interesting Trachynemid were taken in the same locality. Most of them were considerably damaged, and like the specimens of *Aglaura* were devoid of tentacles. Fig. 2 will give a good impression of the medusa. In size the specimens varied from 6 to 10 mm. in broad diameter by slightly more than half as high. Bell subhemispherical in shape with a solid apical projection. Radial canals eight in number, and with gonads borne about the median region on the under side. Manubrium urn-shaped and with flaring, slightly quadrate lips. Color transparent, with an evident iridescence, manubrium dull white, as are also the gonads in formalin specimens. In most respects it agrees fairly well with the description of *Rhopalonema typicum* Maas. from the west coast of Mexico, and later by Agassiz and Mayer from the tropical Pacific.[1]

While the occurrence of the same species in a comparatively high latitude of the Atlantic might seem somewhat improbable, still I am not able to distinguish sufficient differences in specific characters to warrant considering it a new species. As intimated above, the somewhat damaged condition of the specimens and inability to distinguish otocysts may afford characters when determined to justify a different conclusion.

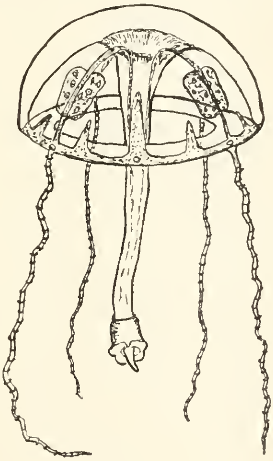

Fig. 3.

In Fig. 3 is shown another medusa not hitherto recorded from the region. As will be seen it bears some resemblance to the

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[1] Cf. Mem. Mus. Comp. Zoöl., Vol. XXVI., No. 3.

Liriope scutigera McCr., but closer inspection will show that in several respects it differs very materially. It would seem to be much closer related to *L. cerassiformis.* Specimens of various sizes showed the various phases in the development and atrophy of the secondary, interradial tentacles, so that in the adult there are only the four perradial tentacles. They were taken in the surface tow near the Gulf Stream. In size the adult specimens were about 10 mm. broad by slightly more than half this height. Bell very transparent, gonads opaque, somewhat shield-shaded, and showing ova in various stages of growth. The centripetal canals were twelve in number, the interradial set being about twice the size of the adradial set. It should be noted that these were exceedingly difficult to distinguish on superficial examination, and this may in part account for their apparent absence in the earlier figures of *L. scutigera.*

In Fig. 4 is shown another medusa taken near No Mans Land

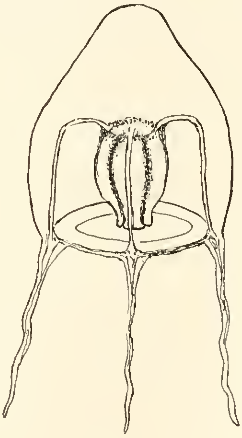

Fig. 4.

on two different occasions during the summer and apparently entirely new to science. Several specimens were taken in the same general locality between that above mentioned and the Gulf Stream. In shape the bell is somewhat oblong, with an extended apical projection. Gastric portion of the manubrium rather large and subquadrate in cross section, mouth simple and with slightly everted lobes ; gonads in four rather prominent masses surrounding the manubrium. Tentacles four, with rather prominent basal bulbs, hollow, attenuate in shape and densely crowded with nematocysts. Velum well developed; ocelli absent; height of bell 2–4 mm. by about half as broad. Color, bell transparent, gonads and manubrium milky white.

While the medusa has characters which indicate relations with the Codonidæ, *e. g.*, the simple mouth opening, straight, simple tentacles, general shape, etc., it has likewise characters which also point to relations with the Tiaridæ, such as the subquadratic manubrium, lobular arrangement of gonads on the radial sides of the manubrium, and the apical projection of the bell. From a critical comparison of the sum of the characters with Hæckel's description of the genus *Protiara* which he established for a medusa which in general characters appears to have much in common with the one under consideration, it seems to find its place appropriately under this generic head, and may be taken to constitute a sort of connecting link between the two families, since Hæckel designates his as the prototype of the Tiaridæ, and most nearly allied to the Sarsiadæ of any of the Tiarids.

But while undoubtedly allied with *Protiara* it is clearly a distinct species, differing in almost all its specific characters, as color, shape, etc. For the present species I propose the name *P. hæckeli*, in honor of the author of the genus under which it falls.

As intimated above several species of Siphonphores were taken in this collection which have not been determined and since they are not at present accessible further account of them will be deferred till such time as they may have adequate description.

In Fig. 5 is shown an interesting Narcomedusa taken also in surface tow near the Gulf Stream. Only a single specimen was taken and this was slightly damaged and apparently immature, having only four perradial tentacles with what seem to be very small interradial tentacles. As in several of the previously mentioned cases, it was not possible to distinguish any sensory bodies. The specimen is a member of the Solmaridæ, and perhaps belongs to the genus *Solmaris*, but in view of its apparent immaturity, indicated by the absence of gonads and undeveloped interradial tentacles, it seems probable that it may be the young of some *Solmaris*. It is perfectly evident that in its present stage of development it does not find close relations with any species at present distinguished. Should further collections of other similar specimens of similar characters justify specific distinctness I would propose for the species the name of *S. tetranema*.

A somewhat rare Scyphomedusa for this region was taken near the Gulf Stream in the locality indicated in the introductory paragraph, namely, *Pelagia cyanella*. So far as I know the only previous record was of a single specimen taken by the "Fish Hawk" in 1899 in essentially the same region.

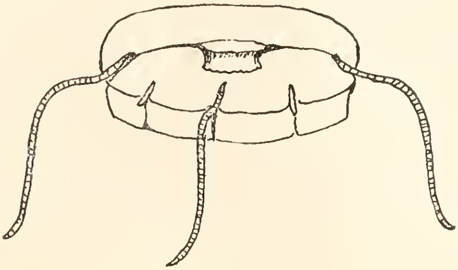

Fig. 5.

The specimen measured about 30 mm. in diameter, being immature, as no marked development of gonads had yet occurred. The previous specimen measured about 50 mm. in diameter and was loaded with well-developed ova apparently ready to be discharged. This is one of the few distinctly pelagic medusæ of this class which occasionally come within the limits of the region, and is at the same time one of the most beautiful,rivalling *Dactylometra*, if not surpassing it in the richness and delicacy of its coloring and grace of form. A fuller description of the medusa will be given in a later contribution.

In this connection may be described an interesting medusa taken in the tow in Great Harbor, Woods Holl, on the evening of August 16. Its general form is well illustrated in Fig. 6. The following brief characters may further differentiate it: Bell subrectangular in profile with a slight apical projection, bell walls rather thin and very transparent, capable of great contraction in both directions; radial canals four, rather wide and simple; manubrium sessile, with broad base and tapering to a terminal oral orifice which is simple and slightly four lobed. Tentacles four, unequally developed, probably due to immaturity. The two on opposite sides (right and left of the figure) presenting the relative size and form, which are quite unique among medusæ of this family (the Codonidæ), though somewhat like greatly exaggerated tentacles of *Dipurena*. In addition to the enlarged terminal knobs which are densely packed with nematocysts, each teniacle has a distinct ring of nematocysts about the middle of the stalk as shown in the figure. In size the medusa measured in life about 2 mm. in height by about 1.5 mm. in breadth. The

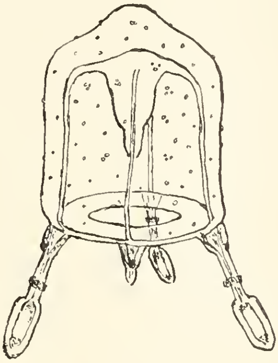

FIG. 6.

exumbrellar surface was irregularly and sparingly dotted with minute clusters of nematocysts. In color the specimen was brilliantly marked, the terminal bulbs of the tentacles being at the apex brilliant carmine tinged with green, and their basal portion duller reddish-brown; the basal bulbs similarly reddish-brown with a dense black ocellus on the outer side. The manubrium was tinged with pale green. It should have been said that the two undeveloped tentacles were not equally so, one being in all but size quite like the larger pair while the smaller one, though much smaller than the other, was evidently lacking in age only to attain to the size and form of the others.

The specimen (only a single one being taken) bears indications of immaturity; *e. g.*, the unequally developed tentacles, absence of distinct gonads, and the nematocysts of the exumbrellar surface. In view of the similarity of swimming habit, coloration, and the general aspect of tentacles, the first thought was of its possibility as the very young of *Dipurena*. A careful study of the bell form, as well as that of the manubrium, and the remarkable development of the tentacular knobs, all are fundamentally unlike *Dipurena*. And while apparently a young specimen it may be doubted whether in all essential respects it is not fairly adult in morphological aspects. I am therefore disposed to regard it as entitled to both generic and specific distinctness, and propose for it, at least provisionally, the name *Dipurella clavata*.

Cruises were made off Nantucket southward on August 13, and off Chatham at "crab ledge" August 19 secured a medusoid fauna in many regards different from that of the former. *Oceania* and *Obelia* were found in about the same numbers as before, but there was a noticeable absence of all those distinctively tropical in their ranges.

On the other hand here were the outliers of an arctic fauna as indicated in such forms as *Pleurobrachia* and *Beroe* among Ctenophores, *Tealia crassicornis*, *Bunodes stella*, among actinians, *Solaster* among starfishes, etc., while of medusæ were found *Hybocodon*, *Corymorpha* and *Trachynema* chiefly.

The occurrence of *Hybocodon prolifer* at this season was quite a surprise, as so far as I am aware its occurrence has been recorded chiefly, if not wholly, during the very early spring. It was in perfect sexual maturity and also budding medusæ proliferously from the base of the tentacle. An interesting condition of these specimens was the absence of the bright orange-red coloration which is so marked a feature of the early spring forms, and with it also the absence of the exumbrellar bands of nematocysts. But for the fact that I have often noted the gradual decline of this feature in late spring specimens I should have felt disposed to consider the specimens taken on this cruise to be of a distinct species. It is not altogether improbable that similar features in the seasonal variation of other medusæ may have been the occasion of thus specifically differentiating one and the same organism.

Here also I took for the first time during the summer the medusa of *Corymorpha pendula*. In general aspects it is much like *Hybocodon* and I am disposed to favor Haeckel's assignment of this medusa to the latter genus. While less marked by asymmetry than is *Hybocodon*, and while having only one perfectly rudimentary tentacle, these do not seem sufficient grounds upon which to establish a distinct genus.

Another interesting medusæ taken at both these stations and apparently a new species, is closely related to *Trachynema digitale*, and was at first considered to be the young of this species. A closer examination, however, showed it to be quite specifically distinct. Fig. 7 will afford a good general impression of the morphological aspects of the medusa. In size it averages about five to six mm. in height, by three mm. in width of bell. This of itself might not justify the conclusion of specific distinction, still as an average of about fifty specimens, taken at considerable distances and at an interval of nearly a fortnight, it would strongly warrant such a probability, when we know the former species to have an average of from twenty-five to thirty-five mm. in bell height by about half that width.

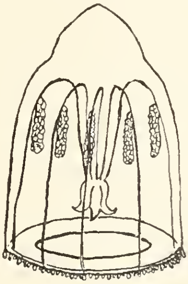

FIG. 7.

Again the color of the former species is quite strongly in contrast with that of the medusa under consideration. In *T. digitale* the bell is said to be light pink, the manubrium reddish and the tentacles at the contracted tips also red. The present species is wholly devoid of color, except for the slightest tint of pinkish iridescence which at times appears under favorable circumstances.

Haeckel has placed Agassiz' species under a different family, the Aglauridæ, and under the new genus *Aglantha*. This readjustment is apparently well founded, and I, therefore, incline to accept it and to propose for the species under consideration the name *Aglantha conica* the specific name designating the somewhat typical cone shape of the medusa.

The following specific diagnosis briefly summarizes the more distinctive characters of the medusa: Bell high with rather sharp apical projection slightly constricted just above the bell cavity. Manubrium long and with elongate gelatinous peduncle; gastric region only about one fourth as long as the former and with prominent four-lobed lips, the whole organ about three fourths as long as the bell cavity. Radial canals eight, extending the length of the peduncle. Gonads eight, cylindrical and suspended from the upper portion of the bell cavity under the canals. Velum well developed and apparently strongly functional as the organ of locomotion; movements of the medusa active and erratic, darting arrow-like when disturbed; bell walls thin with tendency to wrinkle longitudinally when placed in formalin. Tentacles apparently numerous, though mostly lacking, only the bases generally distinguishable; those present were short and rather blunt. No marginal organs or otocysts distinguishable.

The specimens were taken with the open tow-net at a depth of from twelve to sixteen fathoms. Many of the specimens were sexually mature, some females discharging eggs. Both sexes apparently present and in about equal numbers.

These observations would seem in the main to confirm the earlier records of Stimpson, Agassiz, Verrill, Packard and others that the point of Cape Cod marks a limit more or less definite, between a boreal, or "Acadian," and a "Virginian" fauna which pertains to not only cœlenterate life but to a considerable range of invertebrates. Undoubtedly the configuration of the coast line and its associated topography in their influence on the various currents are important factors in the faunal ranges which are under consideration.

In this connection may be discussed another point to which previous reference has been made, namely, the varying depths from which our hauls were made. I regret to say that the nets were only of the ordinary open sort, which therefore precludes anything like exact data, but the repetition of hauls at the same place and from varying depths and a careful comparison of results afford a rough approximation toward an estimate of the relative abundance of life at given depths as compared with the surface, as well as the varying kind of life at the several depths.

In the more open ocean and in the region of the Gulf Stream the larger number and variety were obtained almost entirely from the surface. In the regions of No Mans Land, Nantucket, Chatham Ledge, Vineyard Sound and Buzzard's Bay, on the other hand, a greater abundance and variety of medusæ were obtained from a depth of from 10 to 15 fathoms in water of 17 to 25 fathoms than from either the surface or the bottom. This was more particularly true in rough water and during midday or in bright sunshine. Surface towing during late evening or on dark days most generally give a much larger average in the abundance and variety of species.

In a general way these results, which I have often observed also in previous years, confirm the observations of others and tend to establish what may be considered a law of pelagic life in its relations to light and other aspects of surface environment. At the same time it should not be overlooked, that occasionally there seem to be marked exceptions to such a law. I have taken at times these same forms from the surface in almost incredible abundance, so abundant indeed that with an ordinary pail one might take hundreds at a single dip. Whether sexual conditions, as Agassiz has suggested, or some other condition at present imperfectly known or understood may not be involved it may remain for the future to determine. That sexual conditions *alone* are determining factors seem to me more than doubtful. I should rather incline to consider temperature or tidal currents as probably important factors in the case, just as it seems to me that prevailing winds and currents account for the presence of large numbers of *Aurelia*, *Cyanea*, or other of the Scyphomedusæ, in bays or protected harbors rather than that such segregations are for breeding purposes.

SYRACUSE UNIVERSITY, THE ZOÖLOGICAL LABORATORY,
September 2, 1902.

ON THE MORPHOLOGY OF THE CHROMOSOME GROUP IN BRACHYSTOLA MAGNA.

WALTER S. SUTTON.

The appearance of Boveri's recent remarkable paper[1] on the analysis of the nucleus by means of observations on double-fertilized eggs has prompted me to make a preliminary communication of certain results obtained in a general study of the germ-cells of the great "lubber grasshopper," *Brachystola magna.*

As will appear from a glance at the figures given in my former paper[2] upon the same form, the cells of *Brachystola,* like those of many amphibia, selachians and insects and certain of the flowering plants, exhibit a chromosome group, the members of which show distinct differences in size. Accordingly, one feature of this later study has been a critical examination of large numbers of dividing cells (mainly from the testis) in order to determine whether, as has usually been taken for granted, these differences are merely a matter of chance, or whether, in accordance with the view recently expressed by Montgomery,[3] in regard to a certain pair of elements in the nuclei of one of the Hemiptera, characteristic size-relations are a constant attribute of the chromosomes individually considered. With the aid of camera drawings of the chromosome group in the various cell-generations, I will give below a brief account of the evidence which has led me to adopt the latter conclusion.

In the first generation of secondary spermatogonia, which are the earliest germ-cells I have been able to obtain in *Brachystola,* certain differences in length and volume are to be seen between the members of the chromosome group. These cells, as shown in my former paper already referred to (where they are erroneously described as the last generation of primary spermatogonia), lie in the follicle without definite arrangement and are usually much flattened and distorted by mutual pressure and that of the growing spermatocysts between which they lie. For this reason a study of the chromosome series is difficult in this cell-generation, but, fortunately, I have been able to find a few division-figures which permit of an accurate study of the chromosomes. Such a cell as that shown in Fig. 1—a metaphase in polar view—offers the best opportunities. Here it is apparent at a glance that the chromosomes are of a variety of sizes, but yet in general so nicely graded as to form an almost regular series from smallest to largest. A second glance, however, reveals the fact that there is one very prominent break in this graded series, separating the six smaller chromosomes from the remaining larger ones, and a count of the larger group shows it to contain seventeen units, giving as a total the odd number twenty-three.[1] The odd or twenty-third member of the group, as can be plainly seen in the following division, is the accessory chromosome, which on account of its peculiar behavior will be considered separately. There is, therefore, in the ordinary group, the even number, twenty-two. More especially in the smaller group, but likewise in the members of the sixteen, it can be seen that the gradations in volume are not between individual chomosomes but between pairs, the two members of which in each case are of approximately equal

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[1] Boveri, Th. (1902), "Mehrpolige Mitosen als Mittel zur Analyse des Zellkerns," *Verh. d. Phys. Med. Ges. zu Würzburg,* XXXV.

[2] Sutton, W. S. (1900), "The Spermatogonial Divisions in *Brachystola magna,*" *Kans. Univ. Quart.,* Vol. 9.

[3] Montgomery, T. H., Jr. (1901), "A Study of the Chromosomes of the Germ Cells of the Metazoa," *Trans. Amer. Phil. Soc.,* Vol. XX.

FIG. 1.

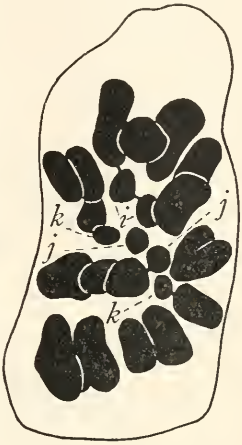

FIG. 1. Polar view of metaphase of first generation of secondary spermatogonia. Six small chromosomes designated by letters *i*, *j* and *k*. (From section.) *Note.*—All figures given in this paper are camera lucida copies of the portions under consideration from the original camera drawings to be published in the forthcoming work to which this is preliminary. The figures are not schematized.

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[1] Montgomery (*l. c.*) has found four of the Hemiptera-heteroptera to possess an odd somatic number of chromosomes and I have observed the same to be true for some fifty species of Acrididæ and Tryxalinæ.

size. In other words, there are, in the ordinary chromosome group, not twenty-two but eleven sizes of chromosomes. The lettering in the figure will indicate the pairs in the smaller group where they are most clearly defined.

Eight[1] generations of spermatogonia follow this one, and in each succeeding metaphase the same number and size-relations of chromosomes may be observed. This is shown in Figs. 2 and 3, representing different secondary spermatogonial generations, in each of which appears six small chromosomes and seventeen larger ones. In each of these also — especially in the case of the smaller group, the members of which, on account of their nearly spherical form, do not suffer the same degree of foreshortening in the drawing as do many of their longer comrades — the paired relation may again be made out. Moreover, in the smaller group with its fewer members and greater size-differences, it is possible to see that the volume of the smallest pair (*kk*), for instance, in one cell bears approximately the same ratio to the homologous pair in another cell as does that of the largest (*jj*) of the

FIG. 2.

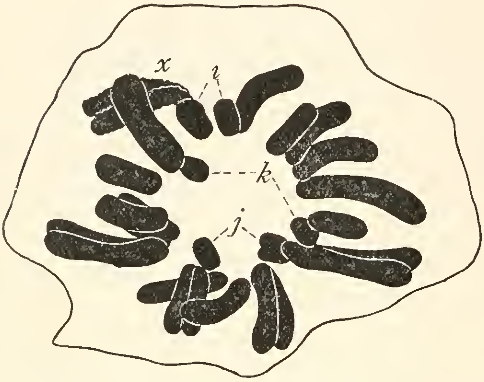

FIG 3.

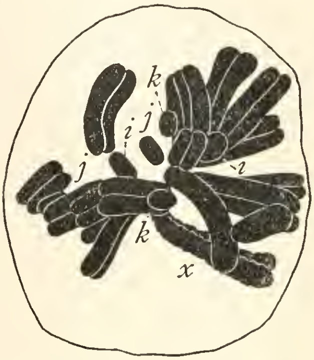

FIG. 2. Polar view of equatorial plate in secondary spermatogonium of one of the earlier generations. (From section.)

FIG. 3. Oblique lateral view of equatorial plate of a secondary spermatogonium of one of the later generations. (From a smear-preparation.) Small chromosomes and accessory designated as in Fig. 1.

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[1] Based on estimates of the number of cells in a spermatocyst at the time of transformation to spermatocytes.

first cell to the largest of the second, or the middle-sized pair (*ii*) of the one to the middle-sized pair of the other. In these cells the compact condition of the chromosomes will not permit of the accurate recognition of individual elements — other than perhaps the largest and the smallest — in the group of sixteen, where size differences are comparatively slight, but this deficiency will be made up in the consideration of the group in the spermatocytes.

Throughout all the secondary spermatogonial generations, in all stages except those of active division, the accessory chromosome remains apart in a vesicle which is virtually a separate nucleus. The genetic relation of the accessory chromosome of any secondary spermatogonium to that of any other in its line of ancestry seems, therefore, unquestionable. Each of the sixteen chromosomes of the larger group has also been enclosed in a separate vesicle (Fig. 4) during the period of metabolic activity, but these vesicles are practically always in communication with one another at their polar extremities, forming there a common compartment in which the six smaller units are frequently found.

FIG. 4.

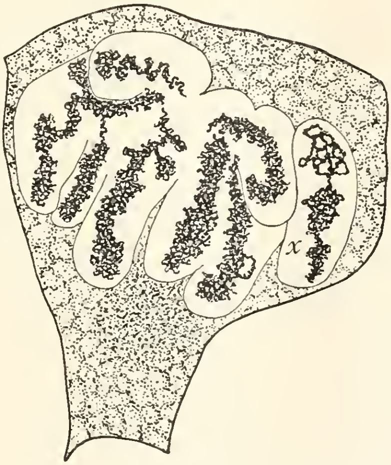

FIG. 4. Secondary spermatogonium in early prophase, showing very fine spiremes arranged in their respective diverticula of the nucleus. Most of the partition walls between diverticula are shorter in the figures than in the preparation, since their crossing if drawn in full would only cause confusion in a line drawing. (From a section.)

In this case there is plainly a possibility for an exchange of chromatic matter; but since each generation exhibits the same series of chromosomes as that before; and since, after the stages of the very fine spireme, the chromosomes reappear one in each sacculation as before, no other conclusion seems credible than that they are, chromosome for chromosome, the same in one generation as in another, just as is the case with the accessory.

During the transformation to spermatocytes, the nucleus as a whole becomes spherical, but, in many cases, the compartments still remain; and in them the chromosomes pass through the fine spireme stages. In this condition, as in that just described for the spermatogonia, it is difficult to conceive the formation of a continuous spireme; but when, at a little later stage, cell and nuclear membranes have become less resistant so that their contents may be smeared upon cover-glasses and there fixed for study *in toto*, it becomes clear that fewer spiremes are present than in the spermatogonial nuclei. In every case, the accessory chromosome appears in its peculiar characteristic condition (*x*, Figs. 5 *a*, 5 *b*, 6 and 7), and careful counting of a large number of cases *shows the spiremes in every favorable instance to number eleven.* These spiremes are graded as to size just as were the chromosome-pairs of the spermatogonia; and the gap in the series separates a group of *eight large* from a group of *three small* elements. In most of the spiremes a longitudinal split is clearly visible, and in addition, in practically every case, a division may be noted separating the spireme into two distinct limbs of approximately equal size, which are frequently doubled on each other at the point of union.

If now we seek the relation of these spiremes to the chromosomes of the spermatogonia, we find abundant data. Twenty-two chromosomes enclosed in separate compartments, each opening at one end into a common chamber, are represented by eleven double chromosomes. Scarcely any two of the eleven are even approximately of the same size, whereas each of the twenty-two appeared to have a mate of like volume. But the eleven double chromosomes are made up each of two limbs of equal size and we find it difficult to believe that these limbs do not represent the members of the pairs, joined together at their polar ends,

FIG. 5a.

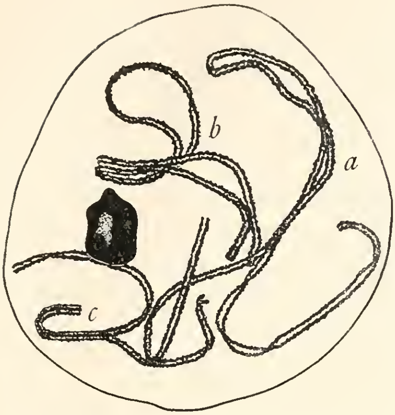

FIG. 5b.

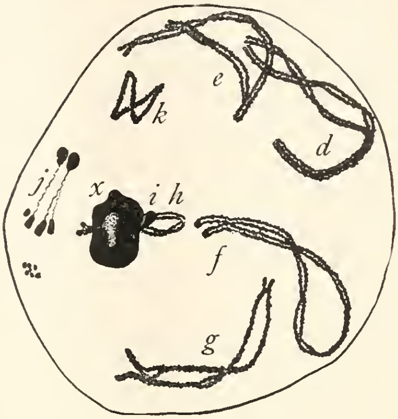

FIG. 5a. Three largest spiremes and accessory chromosome from a primary spermatocyte in early prophase. (Smear-preparation.)

FIG. 5b. Eight remaining chromosomes and accessory from same cell as Fig. 5a.

FIG. 6.

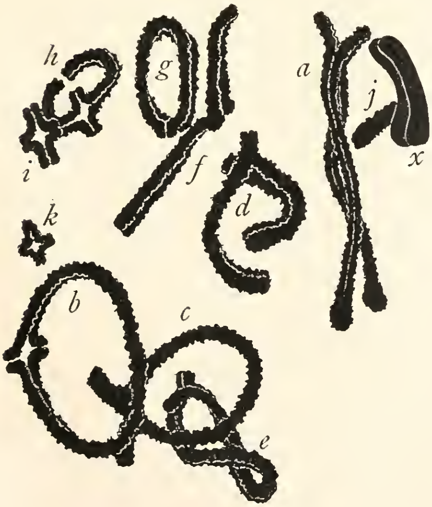

FIG. 7.

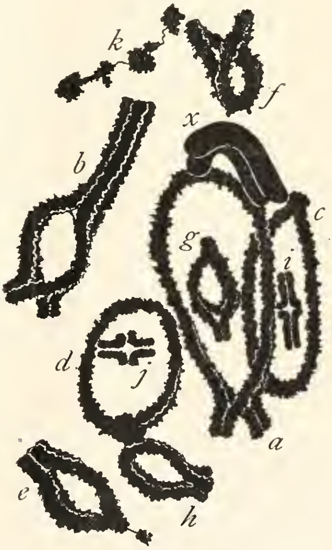

FIG. 6. Partly condensed spiremes in middle prophase of primary spermatocyte. All the chromosomes, including the accessory (*x*), show indications of a longitudinal split.

FIG. 7. Slightly more advanced chromosome group than that of Fig. 6. Letters *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*, *j* and *k* designate the different chromosomes in order of size from largest to smallest; *x* designates the accessory.

which, as we have seen, projected into a common chamber of the nucleus. To such a conclusion additional weight is added by the occasional finding of telophases of the last spermatogonial generation which actually shows such a fusion (Fig. 8).

FIG. 8.

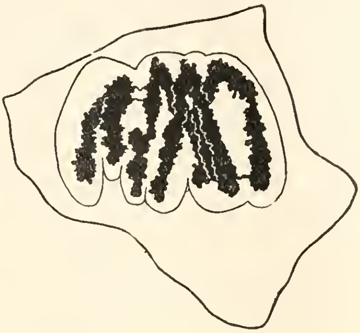

FIG. 8. Telophase of secondary spermatogonium of the last generation showing synapsis. Only a few of the chromosomes are shown and as in Fig. 4; to avoid confusion the sacculations are drawn only to the point where they cross one another.

The four parts of each spireme, marked off by the longitudinal split and the line of fusion, may be traced through all the prophases up to the metaphase, where they are clearly seen to become the four parts of the tetrad. These facts seem to me to leave no escape from the conclusion that in the completed tetrad the longitudinal split represents merely the usual division of a chromosome into equivalent chromatids; but *the transverse marking separates two spermatogonial chromosomes which have conjugated end-to-end in synapsis.*[1]

Notwithstanding the fact that no continuous spireme is formed, the various spiremes of the larger group (16 in spermatogonia, 8 in spermatocytes) in any nucleus are at any given period always of approximately the same diameter and the same degree of concentration. Their respective lengths may therefore be taken as a measure of their respective volumes, and accordingly the longer and more slender the spiremes, the more pronounced their differences of volume would appear. Obviously, it is impossible to study the length of convoluted spiremes in sections. Smear-preparations also fail in the spermatogonia on account of the strength of the nuclear membrane, which in these cells resists the roughest treatment and prevents the separation of its contained spiremes. But in the prophases of the primary spermatocytes the nuclear membrane becomes so thin and weak that its contents may be readily smeared upon a cover-glass and the spiremes thus separated and to a certain extent flattened in the plane of the cover. In the most favorable of these cases, such as those shown in Figs. 5, 6 and 7, which represent different stages in the concentration of the spermatocyte spiremes, a more or less accurate comparison by means of measurements is possible. For the sake of convenience in reference, we will designate the chromosomes in these figures by the first eleven letters of the alphabet, beginning with the longest chromosome and proceeding according to size. The chromosomes as drawn are in all cases simple projections and hence suffer a greater or less amount of foreshortening according to the degree of their curvature or inclination to the plane of the slide. This, however, is so slight that it has been disregarded in the table except in case of chromosomes *b* and *h* of Fig. 6. In these cases, the actual length in the figure is given in parentheses and an estimate of the real length in the regular column. No attempt was made to measure the three smaller elements, as their variations in form and diameter in the spermatocytes render measurement in one

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[1] Cf. Montgomery, T. H., Jr. (1901), "The Spermatogenesis of *Peripatus* (*Peripatopsis*) *Balfouri* up to the Formation of the Spermatid," *Zool. Jahrb.*, XV.; also "A Study of the Chromosomes of the Germ Cells of the Metazoa," *Trans. Amer. Phil. Soc.*, Vol. XX.

	Fig. 5.	Fig. 6.	Fig. 7.
a	43	22	21
b	32½	19½(17)	17
c	23	15½	15
d	20	14	12
e	17½	12½	9
f	16½	10⅔	7⅔
g	15	9	7½
h	(7½)	8½(7⅔)	7
i			
j			
k			

NOTE. — The figures are in terms of an arbitrary unit equivalent to the distance apart of the divider-points used in making the measurements.

dimension of no value whatever. Naturally these figures can make no pretensions to complete accuracy but as approximations they serve to show a uniformity in the different nuclei that cannot justly be ascribed to chance. It is worthy of note that the only case in which a chromosome does not bear approximately the same ratio as its mates to the homologous members of the other two series is chromosome *h* of Fig. 5; which being hidden for the most part behind the accessory, is at best a doubtful quantity.

When the ordinary chromosomes divide in the first mitosis of the spermatocytes, the separation takes place along the line of the longitudinal split and therefore, except that the chromosomes are joined together by pairs, differs in no respect from an ordinary spermatogonial division. The accessory chromosome, however, though showing a clearly-defined longitudinal split, *does not divide but passes entire to one pole*, as Sinéty[1] has independently observed in the Phasmidæ; and after completion of the division may be clearly seen in one only of the two daughter cells, where it is sharply contrasted with the partially disintegrated ordinary chromosomes.

Fig. 9.

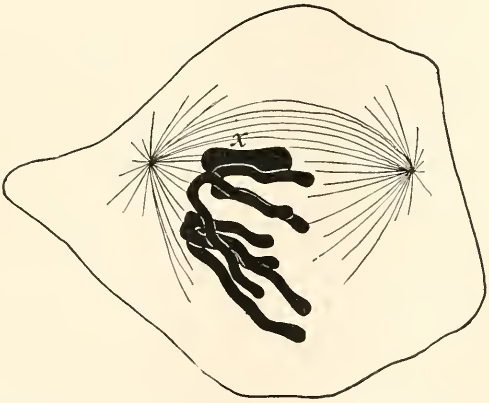

Fig. 9. Four ordinary chromosomes and accessory from a very late prophase of the secondary spermatocyte division. Each ordinary chromosome is made up of two limbs connected at one end. There is no longitudinal split.

In the prophases of the second division the chromosomes reappear in the same number and show the same size relation as in the preceding telophase, but instead of exhibiting a longitudinal split *they are seen to be composed of two equal limbs joined together at one end only* (Fig. 9) *just as when they passed to the pole in the previous anaphases.* The division occurs at the point of junction of the two limbs and is unquestionably transverse — *separating the two chromosomes at the point where they fused in synapsis two generations before.*

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[1] Sinéty, R. de (1901), "Recherches sur la biologie et l'Anatomie des Phasmes," *La Cellule*, T. XIX.

In those secondary spermatocytes in which the accessory chromosome occurs, this element also divides, but in the line of the longitudinal split which has persisted from the prophases of the primary generation. One half of the resulting spermatids, therefore, are characterized by the presence of the accessory and the other half by its absence, but this constitutes the only morphological difference between the two categories. In each, the ordinary chromosomes may be seen to constitute a graded series of eleven members in which a considerable gap at one point separates a subgroup of three small units from another subgroup of eight larger ones (Fig. 10). The series therefore represent exactly half of the ordinary chromosome group as we found it throughout the secondary spermatogonia. This is the chro-

FIG. 10.

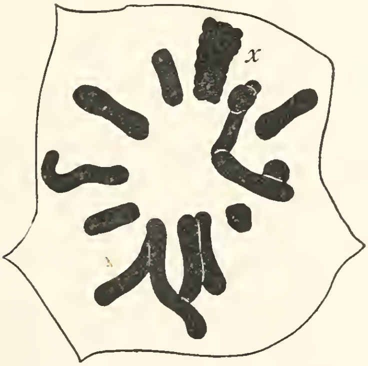

FIG. 11.

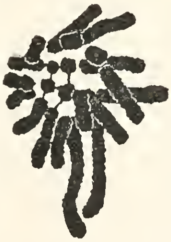

FIG. 10. Polar view of chromosome group in metaphase of secondary spermatocyte containing the accessory chromosome. Eight large and three small ordinary chromosomes appear. (From section.)

FIG. 11. Axial view of chromosome group of ovarian follicle-cell in telophase. Twenty-two chromosomes appear of which six are decidedly small and the other sixteen decidedly larger.

mosome series which forms the chromatic portion of the sperm-head and consequently is to be regarded *a priori* as the series which will reappear in the sperm-nucleus in the act of fertilization. If this is true — and everything speaks for and nothing definitely against the correctness of the assumption — then the conclusion seems unavoidable that the mate to each of the eleven chromosomes must be furnished by the egg-nucleus to produce the eleven pairs characteristic of all the early germ-cells, of the follicle-cells of the ovary as shown by Fig. 11, and probably also of the ordinary somatic cells.[1]

To sum up, in *Brachystola* the nuclei, not only of the presynaptic germ-cells, but also of cells which have been shunted off from the germinal cycle, are characterized by the possession of a chromosome group made up of two morphologically equivalent series of eleven members each.[2] Comparison shows that the size-relations between various members of these series are approximately the same in different nuclei of the same or different cell generations. The numerical reduction (pseudo-reduction) is accomplished by the union of homologous members of the two series of a nucleus, and this union is terminated in the second spermatocyte division by the separation of the daughter-chromosomes of the original conjoints at their point of union and their passage to opposite poles. We are virtually able to recognize each chromosome in eleven consecutive cell-generations; and in the prophases and telophases of nine of these, the chromosomes are separated from one another for a great part of their length, only their polar ends lying in the common chamber. No continuous spireme is formed; and although after each division there is a brief interval, during which chromosomic boundaries can no longer be traced, the regular correspondence, unit for unit, of the mother series with the daughter series establishes a high probability that we are dealing with morphologically distinct individuals, each of which bears to its mother element a genetic relation comparable to that existing between mother- and daughter-cells.

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[1] Cases of cells other than germ-cells in which an accurate count of the chromosomes is possible are extremely rare on account of the crowded condition of their nuclei; but I am able to state that in the cells of the ovarian follicles and of the common collecting ducts of the testes every division figure shows large and small chromosomes in apparently the same relation as those found in the spermatogonia.

[2] Besides the accessory chromosome.

I have endeavored to show that the eleven ordinary chromosomes which enter the nucleus of each spermatid are selected one from each of the eleven pairs which made up the double series of the spermatogonia. It now becomes a highly interesting question whether there exists in the ripe egg a graded series of chromosomes similar to that of the mature male element. I have found the chromosome group not only of the oögonia but also of the ovarian follicle cells (Fig. 11) to correspond perfectly with that of the spermatogonia ; and if we are permitted to assume that the reduction process in both sexes is the same, we have no alternative but to believe that the chromosome series of the mature germinal products also are alike. Obviously, copulation of such nuclei in fertilization would restore the conditions which we have found not only in the early germ-cells but in some outside the line of succession.

These latter observations have totally disregarded the accessory chromosome, but it is in it, if further research shall substantiate my present limited but thoroughly consistent results, that we shall find our most unequivocal evidence of chromosomic individuality. We have noted that the spermatogonia have twenty-three chromosomes, and that the odd one of these is the accessory which by means of its idiosyncrasies may readily be recognized in all except the active mitotic stages. We have also noticed that this element is unequally distributed in the maturation divisions and as a consequence occurs in exactly half the spermatozoa. In the oögonia and ovarian follicle-cells in which I have been able to count the chromosomes, I have found but twenty-two ; and the fact that none of these behaves in the characteristic manner of the accessory proclaims it the missing member.

We should expect therefore to find but one kind of mature ova in respect to number of chromosomes while we know that by the same standard there are two kinds of spermatozoa. Obviously, then, the number of chromosomes in the cleavage-nucleus of the fertilized egg (twenty-two or twenty-three) must depend upon the number introduced in the sperm-nucleus, since the latter contains either eleven or twelve, according as the accessory chromosome is absent or present. Now twenty-three is the number of chromosomes in the male cells, while twenty-two is the number I have found in the female cells, and thus we seem to find a confirmation of McClung's[1] suggestion that the accessory chromosome is in some way concerned in the determination of sex.

Without discussing here the logical consequences of such a conclusion, I will only emphasize the fact that one of the chromosomes, which in the primary spermatogonia[2] is scarcely distinguishable from its fellows, maintains throughout a long series of divisions an indubitable independence; and finally completely establishes its right to the title of a distinct individual by passing entire to one daughter-cell with the result that no accessory chromosome appears in the products of the next division of the other.

Taken as a whole, the evidence presented by the cells of *Brachystola* is such as to lend great weight to the conclusion that a chromosome may exist only by virtue of direct descent by longitudinal division from a preëxisting chromosome and that the members of the daughter group bear to one another the same respective relations as did those of the mother group — in other words, that the chromosome in *Brachystola* is a distinct morphological individual.

This conclusion inevitably raises the question whether there is also a physiological individuality, *i. e.*, whether the chromosomes represent respectively different series or groups of qualities or whether there are merely different-sized aggregations of the same material and, therefore, qualitatively alike.

On this question my observations do not furnish direct evidence. But it is *a priori* improbable that the constant morphological differences we have seen should exist except by virtue of more fundamental differences of which they are an expression; and, further, by the unequal distribution of the accessory chromosome we are enabled to compare the developmental possibilities of cells containing it with those of cells which do not. Granting the normal constitution of the female cells examined and the similarity of the reduction process in the two sexes, such a comparison

[1] McClung, C. E., "Notes in the Accessory Chromosome," *Anal. Anz.*, XX.; "The Accessory Chromosome, Sex-determinant," BIOL. BULL., III.

[2] A study of the chromosomes of the primary spermatogonia has been made in *Melanoplus differentialis*, a nearly related form in which the later divisions are essentially the same as in *Brachystola*.

must show that this particular chromosome does possess a power not inherent in any of the others — the power of impressing on the containing cell the stamp of maleness, in accordance with McClung's hypothesis.

The evidence advanced in the case of the ordinary chromosomes is obviously more in the nature of suggestion than of proof, but it is offered in this connection as a morphological complement to the beautiful experimental researches of Boveri[1] already referred to. In this paper Boveri shows how he has artificially accomplished for the various chromosomes of the sea-urchin, the same result that nature is constantly giving us in the case of the accessory chromosome of the Orthoptera. He has been able to produce and to study the development of blastomeres lacking certain of the chromosomes of the normal series.[2]

In larvæ resulting from double-fertilized eggs which have divided into three cells at the first cleavage, he recognizes an organism made up of definite thirds, each traceable to one of the original blastomeres and each characterized, as a result of the primary hap-hazard tripolar division, by a different combination and generally by a different number of chromosomes from that of its fellows. In rare instances such an embryo may be normal, of which fact the possibility that each pole of the triaster may receive a complete normal series of chromosomes is explanation enough. In other cases, the embryo may be completely normal (for instance in respect to skeleton or pigmentation) in one or two thirds, while in the remaining portion these structures may be entirely lacking; and it is a most significant fact that "in einzelnen dieser Fälle konnte aus der Kerngrösse nachgewiesen werden, dass die Grenze wo der Defekt beginnt, mit der Grenze zweier auf verschiedene Ausgangsblastomeren zurückführender Bereiche zusammenfällt." In the "normal" embryos mentioned above, every part was present, and as regards specific characters, normal; but in different thirds there could be seen individual variations which normally should have appeared in different larvæ. "In der

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[1] Boveri, *l. c.*

[2] By the normal series is here meant such a one as occurs in the nucleus of either of the mature germinal products, since it has been clearly shown by the well-known work on the fertilization of enucleate egg-fragments and on chemically induced parthenogenesis, that either of the ripe germ-products possesses all the chromatin necessary for the production of a normal larva.

That," says Boveri, "könnte ich aus den verschiedenen Typen der normalen Kontrolzuchten, durch Kombination der rechten Hälfte einer Larve mit der linken einer anderen, Bilder herstellen, die den in Rede stehenden Dreierplutei fast genau entsprechen." To these points is added the fact that while all the isolated blastomeres of a normal four-cell stage develop exactly alike, those of dispermic three- or four-cell stages rarely or never do so, even when the numerical distribution of chromosomes appears equal; and, further, that in large numbers of larvæ from double-fertilized eggs all possible combinations of characters are to be found, just as all possible combinations of chromosomes from the three parent cells may enter into the composition of their nuclei. From these and other data, Boveri draws the conclusion that "Nicht eine bestimmte Zahl sondern eine bestimmte Kombination von Chromosomen zur normalen Entwicklung notwendig ist, und dieses bedeutet nichts anders als dass die einzeln Chromosomen verschiedenen Qualitäten besitzen müssen."

Thus we are brought to recognize a physiological individuality in a form in which the chromosomes are morphologically indistinguishable and the nuclei of which, after the anaphases, offer no mechanical hindrance to the free intermixture of the chromatin. We have already reviewed the reasons for believing the accessory chromosome in the cells of *Brachystola* to be the possessor of specific functions and it only remains again to call attention to the likelihood that the constant morphological differences between the ordinary chromosomes are the visible expression of physiological or qualitative differences.

In conclusion, from the point of view thus suggested, let us again consider the phenomena of fertilization. In either sperm- or egg-nucleus a complete series must be present since either may produce a normal embryo without the other. Every normal fertilized egg, therefore, as well as every cleavage-cell derived from it, must have the field of each character covered by two chromosomes — one from each parent. The chromosome series of the echinoderm cleavage-nucleus is thus shown to be physiologically a double one just as in *Brachystola* we have seen it to be morphologically double, and the doubling in both cases is seen to be accomplished in an exactly similar way — viz., by the contribution of equivalent series by the two parents.

If, as the facts in *Brachystola* so strongly suggest, the chromosomes are persistent individuals in the sense that each bears a genetic relation to one only of the previous generation, the probability must be accepted that each represents the same qualities as its parent element. A given relative size may therefore be taken as characteristic of the physical basis of a certain definite set of qualities. But each element of the chromosome series of the spermatozoon has a morphological counterpart in that of the mature egg and from this it follows that the two cover the same field in development. When the two copulate, therefore, in synapsis[1] the entire chromatin basis of a certain set of qualities inherited from the two parents is localized for the first and only time in a single continuous chromatin mass; and when in the second spermatocyte division, the two parts are again separated, one goes entire to each pole contributing to the daughter-cells the corresponding group of qualities from the paternal or the maternal stock as the case may be.

There is, therefore, in *Brachystola* no qualitative division of chromosomes but only a separation of the two members of a pair which, while coexisting in a single nucleus, may be regarded as jointly controlling certain restricted portions of the development of the individual. By the light of this conception we are enabled to see an explanation of that hitherto problematical process, synapsis, in the provision which it makes that the two chromosomes representing the same specific characters shall in no case enter the nucleus of a single spermatid or mature egg.

I may finally call attention to the probability that the association of paternal and maternal chromosomes in pairs and their subsequent separation during the reducing division as indicated above may constitute the physical basis of the Mendelian law of heredity. To this subject I hope soon to return in another place.

I take pleasure in expressing here my gratitude to Prof. E. B. Wilson for much valuable advice and assistance in the work upon *Brachystola* and in the preparation of the present paper.

Zoölogical Laboratory, Columbia University,
October 17, 1902.

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[1] The suggestion that maternal chromosomes unite with paternal ones in synapsis was first made by Montgomery (1901, I.).

THE NERVOUS SYSTEM IN GONIONEMA MURBACHII.

IDA H. HYDE.

Some physiological work which I undertook this summer at the Woods Hole Biological Laboratory on the Hydromedusa, Gonionema Murbachii, made it important that I should know the distribution of its nervous system which at the time had not been described or known with certainty. I decided, therefore, to gain some knowledge of the distribution of its nerves by means of Bethe's methylene-blue method. Inasmuch as my time for this work at Woods Hole was very limited, I was unable to undertake an exhaustive histological study of the whole nervous system of the medusa. Believing it of some interest to make known the facts that were obtained, it was decided to publish them in a brief preliminary report, hoping in the near future to see the subject completed by Mr. Chas. G. Rogers who has consented to make a detailed histological study of the distribution of the nerves, in connection with some work which he is pursuing on regeneration in this animal, under the direction of Dr. Loeb.

All my observations were made on fresh material. The whole or small parts of the animal were exposed six or more hours to the action of a weak sea-water solution of Bx methylene blue freshly made and filtered for each study. The material was kept in a cool place and transferred for study from methylene blue to sea-water or a *m*/8 solution of sodium chloride. Some material was kept for future study as long as twenty-four hours on ice in 10 per cent. ammonium molybdate to which a few drops of 1 per cent. osmic acid and hydrogen peroxide had been added (1 gm. ammonium molybdate, 10 c.c. distilled water, 1 c.c. hydrogen peroxide, two drops 1 per cent. osmic acid).

Small pieces were dissected from different regions and examined with an oil immersion lens. The study of these sections disclosed, besides the double nerve ring around the margin; usually designated as the central nervous system, a third ill-defined very narrow nervous strand, peripheral to the above, around the margin; a definitely outlined radial system along the radial canals, both below the epithelial cells of the subumbrella as well as deeper beyond the muscle layer, in the flat epithelial endo-

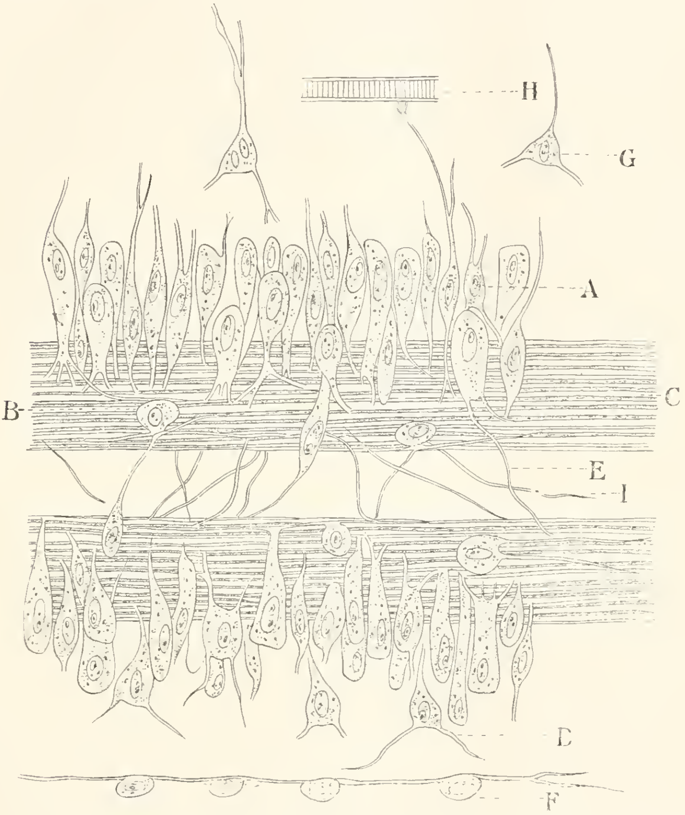

FIG. 1. Schematic view of a piece of the marginal nerve rings. Drawn from a study of many specimens studied with an oil immersion as well as lower power and camera lucida both from the sub- and exumbrella sides.

A, cells, transversely to the upper marginal ring.
B, ovoid cells with sheathed fibers.
C, nerve fibers parallel to the marginal ring.
D, multipolar cells proximal to the lower ring cells.
E, communicating fibers between the rings.
F, ovoid cells in the marginal strand.
G, multipolar cell of the subepithelial network.
H, muscle fiber.
I, space between upper and lower ring.

dermal layer; and a ganglionic and fibrous network beneath the epithelial layer of the subumbrella, which I believe sent nerve fibers beyond the endodermal flat epithelial-like cells, up to the gelatinous layer. The manubrium contains a ganglionic and fibrous network connected with large ganglia that lie along the radial area and smaller ones around its margin that send fibers to small sensory cells at its periphery, (Fig. 3).

A view from the exumbrella side of the margin of the animal discloses a narrow hyaline-like area between the marginal nerve rings (Fig. 1, *i*). It is along this line that the velum is attached. The upper nerve ring lies therefore above, the lower below the attachment of the velum. They are, however, not entirely distinct but are connected by communicating fibers that show most clearly, with proper focusing, from the subumbrella side (Fig. 1, *e*). The nerve rings are composed first of several rows of closely packed bi- and multipolar cells that lie tranversely to the nerve rings beneath the epithelial layer of the subumbrella (Fig. 1, *a*). They are very characteristic elongated cells with large nuclei. They usually taper at one end, more than at the other, and give off one or more fibers from the ends. Some of the fibers extend into the velum, some bend and run at a deeper level along with other nerve fibers around the margin, forming a double fibrous ring (Fig. 1, *c*). Some fibers extend into the other nerve ring, while still others extend toward the apex of the bell communicating either with the nervous network of the subumbrella, the radial nerves, or ending in muscle fibers. In addition to these snugly-packed spindle or ovoid-shaped cells, there are bipolar or multipolar cells, that lie at a deeper level in the fibrous portion of the ring canal (Fig. 1, *b*). The fibers emerge from the same side of the bulging or ovoid cells and extend in opposite directions among the fibers of the nerve ring (Fig. 1, *b*). At close intervals and proximal to the transversely situated cells of the lower marginal ring are, moreover, large multipolar cells that send fibers toward the periphery of the margin as well as into the nerve ring. A row of flattened ovoid bipolar cells form a third nerve ring of fibers and cells peripheral to those described above (Fig. 1, *f*). Pieces of tissue dissected from the umbrella in the region of the radial canals and examined both before and after removal of the genital glands, the cœlenteric canal or overlying gelatinous layer, show from the subumbrella side, at the border of the radial canal, closely arranged bi- and multipolar cells (Fig. 2, *k*, *j*) and focusing a little deeper a band of fine parallel nerve fibers intermingled with ovoid nerve cells that send off thicker fibers (Fig. 2, *j*, *l*). Focusing still deeper, beyond the radial muscle fibers, somewhat smaller bi- and multipolar cells come to view (Fig. 2, *s*, *t*). The latter can best be seen from the exumbrella side. The first kind of cells lie below the ectodermal epithelium of the subumbrella. Some of their fibers extend laterally, seemingly to the muscles or the subumbrella network of ganglia and fibers (Fig. 2, *j*), others

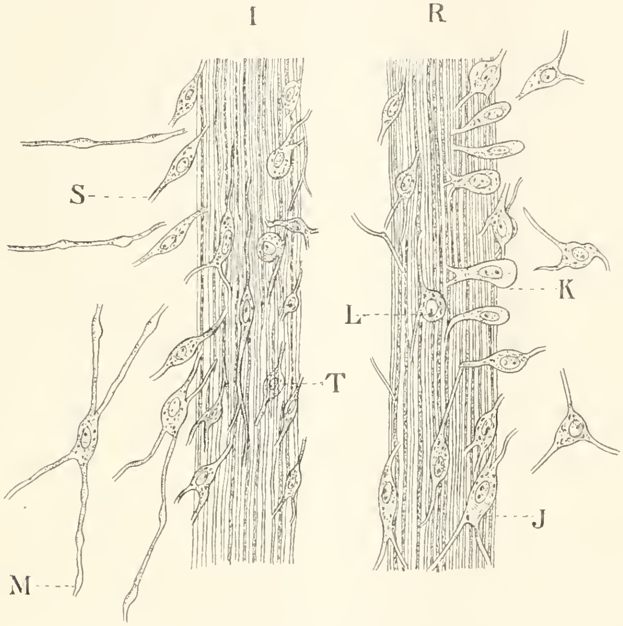

FIG. 2. Schematic view of a piece of the radial nerve tissue. The band at the right (*R*) represents the nerve cells below the ectodermal epithelium of the subumbrella; at the left (*I*) those between the muscles and the endodermal cells as seen from the exumbrella side.

J, multipolar cells, close to radial nerve fibers.
K, radial tissue cells.
L, ovoid cells among the radial nerve fibers.
M, beaded fiber of subepithelial network.

go to the band of parallel nerve fibers and the nerve ring. These cells are more numerous in the neighborhood of the latter. Studied from the exumbrella side it was seen that the parallel nerve fibers lay close to the radial muscle fibers, and that besides the bi- and multipolar cells beneath the ectodermal epithelium, there were other, smaller, bi- and multipolar cells, situated between the muscle layer and the endodermal epithelium.

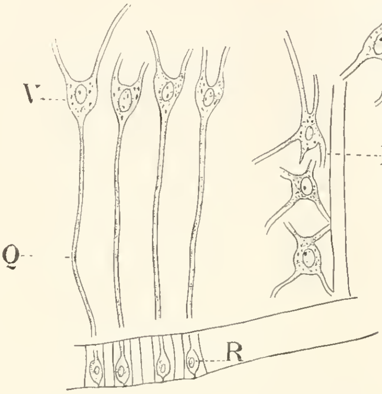

FIG. 3. Schematic view of nerve cells in a piece of the manubrium from its margin in the radial region.

P, multipolar cells in radial region of manubrium.
Q, fibers from multipolar cells at border of manubrium.
R, sensory cells in epithelium of manubrium.
V, multipolar cells at the border of manubrium.

The fibers from these cells seem to go to the genital glands, the cœlenteric canal and narrow band of muscle fibers beneath the radial cœlenteric canal, as well as into the parallel layer of nerve fibers. It may be that these are especially concerned with the digestive and reproductive systems.

In addition, therefore, to the marginal nerve ring and the radial nervous system, there is the peripheral network of cells and fibers that exist beneath the epithelium of the subumbrella. The cells of this network are triangular and multipolar with sheathed fibers that have slight thickenings at different places in their extent. Some lie close to the radial (Fig. 2, *m*), others to the marginal nerve bands (Fig. 1, *g*). Then, too, in a line midway between the radial nerves, the cells lie radially closer, sending some of their fibers up to the gelatinous layer. This net-work thus serves as a connecting link between the radial and marginal nervous structures. The manubrium also has its network of cells and fibers and along the margin of the manubrium is a row of large multipolar cells (Fig. 3, *v*) that send fibers to the periphery where they join special sensory cells that lie among the epithelial cells (Fig. 3, *r*). In the radial region of the manubrium are larger closely set nerve cells. It was seen that fibers from the nerve rings extend to the tentacles and sense organs, and that the latter contained most peculiar nerve tissue. To understand that and the endings of the different nerve fibers, requires detailed histological study of sections of the various regions of the body. This I hope will be done in the near future.

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BIOLOGICAL BULLETIN.

THE HABITS OF CALIFORNIA TERMITES.

HAROLD HEATH.

Situation of Nest. — Of the six species of termites inhabiting the western part of the United States, three, *Termopsis angusticollis* Walk., *Calotermes castaneus* Burmeist. and *Termes lucifugus* Rossi, occur within one hundred miles of San Francisco. The first named is by far the largest and is seemingly the most abundant. At various points, for example the pine woods not far distant from the Hopkins Seaside Laboratory at Pacific Grove, colonies may be found in almost every stump and decaying log and even dead branches on otherwise healthy trees are frequently infested. As in the case of several other termites their excavations follow more or less closely the grain of the wood and are confined to its deeper portions, leaving an outer protective hull, perforated at various points by tunnels leading from the interior to extensive spaces beneath the bark where the eggs and young are housed during the warmer hours of the day. No galleries lead from the nest into the earth nor into adjacent regions as is the case with *Termes* and if for any reason a portion of a log containing the royal pair be separated even by a few inches from the remainder no intercommunication ever takes place and the latter in due time produces substitute royal forms which maintain the community.

Calotermes castaneus, scarcely more than half as long as the above-described species, appears to be comparatively rare in the central portions of the state. I have taken but two nests, one at Pacific Grove and another on the Stanford University campus, the first in a pine log also inhabited by *Termopsis* though the tunnels of the two did not communicate, the second in a eucalyptus stump. Judging from these two colonies their habits are essentially the same as those of *Termopsis* though their movements are executed with greater deliberation.

Termes lucifugus, possibly introduced from Europe, has already been the subject of several papers, notably those of Lespes[1] and Grassi and Sandias,[2] and I have only a few observations to add. In California this species is almost as abundant as *Termopsis* and inhabits indifferently several species of oak, conifer and eucalyptus and is sometimes resident in orchard trees and vineyards. Occasionally it attacks the timbers of houses where the moisture is sufficient though its ravages are not serious. During the dry season from June to October, they frequently abandon their haunts above ground and, by means of subterranean tunnels, depart into more favorable regions. On several occasions I have seen them three or four feet beneath the surface and I am told that in some of the more arid sections of the state they sink their shafts to twice this depth. Under such circumstances the burrow does not follow roots as Grassi considers is the case with the European form, but plunges directly into the soil. This and other peculiarities connected with the burrowing habits of this species may be readily observed if a number of individuals are placed in a tall glass jar filled with earth and chips of wood. Almost immediately they commence to tunnel, removing bits of sand and earth to the surface or storing them in cracks with the rapidity of the true ants. Within a week the whole mass of soil is penetrated by a perfect labyrinth of tunnels, which in many places where the walls are in danger of falling in are lined with excrement. In old burrows the walls throughout possess this lining.

In addition to those galleries which pass downward into regions of sufficient moisture there are others that traverse the earth more or less parallel with its surface and sometimes, in the form of tunnels built of particles of wood, come above ground, where they are concealed under sticks and leaves. In one colony inhabiting a large root of the yellow lupine (*Lupinus arboreus*) in a patch of loamy soil in a rocky, mountainous district not far from San Jose at least a dozen such tubes existed. One of these, occasionally rising to the surface, led to another root nearly twenty feet away. Another extended in an opposite direction a little over thirteen feet, while some of the other tunnels traversed shorter distances. From these outlying posts other galleries extended and, so far as it was possible to judge, all the roots in that area, nearly an acre in extent, were inhabited by one extensive colony.

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[1] "Recherches sur l'organisation et les moeurs du Termite lucifuge," *Ann. Sci. Nat.* (4), V. (1856), pp. 227–282, pls. V.–VII.

[2] "Costituzione e Sviluppo della Società dei Termitidi," *Alti deu' Aaccademia gloenia di Scienze Naturali in Catania*, Ser. 4, Vols. VI. and VII. (1893–4). Translation by W. F. Blandford in *Quart. Jour. Mic. Sci.*, Vols. XXXIX. and XL. (1897–8).

Number of Individuals in the Colony. — In such cases and even more where the areas are less circumscribed it becomes practically impossible to determine the limits of a community and the number of members composing it. From this colony just described I took 3,761 individuals — a number probably less than half. On another occasion I took over 1,800 winged forms as they were swarming from a hole almost in the middle of one of the much-travelled streets of Palo Alto. If it be true that the sexes of this species swarm at different times, as Grassi maintains, it becomes evident, in connection with the fact that the winged individuals compose considerably less than half of the colony, that the latter consists, in some cases at least, of over 6,000 inhabitants.

With *Termopsis* the number of individuals is less than in the above described cases. Where the colony is accompanied by the primary royal pair and has been in existence for from one to three and perhaps four or five years its members number from 50 to 1,000. After the death of the primary pair and several substitute royal forms have been developed the egg-laying process is relatively much more rapid and results in the formation of large communities. One such, inhabiting a huge pine log, numbered 3,221 and judging from the thousands of eggs deposited throughout the burrows this number must soon have been greatly exceeded. Under average conditions, however, 2,000 is probably about the usual number.

The nests of *Calotermes* were both small, in one case aggregating about 200 individuals, in the other nearly twice this number. These contained neither eggs nor young and were located in almost completely decayed wood and hence may not be perfectly typical.

Swarming and Founding of Colony. — As is well known, the usual termite colony consists of a royal pair, numerous soldiers, workers (absent in *Calotermes*) and perfect insects together with larvæ in all stages of growth. The peculiarities of these castes in the California species will be discussed in connection with their breeding habits and the establishment of new colonies.

In the case of *Termopsis* the nymphs (larvæ of perfect insects with plainly visible wing buds) that have developed during the year, undergo their last molt in from ten to twenty minutes, sometimes in the late summer or early autumn, unfold their wings during the next two hours and within a fortnight have changed from relatively helpless individuals to the active, dark-hued insects ready for their extended nuptial flight. This takes place just at dusk and is preceded by a period of great restlessness. From openings which have been made for them by the workers they issue rapidly by twos and threes and, with scarcely any preliminary movements, rise into the air. Some soon fall to the ground but in an open country others may continue their flight fully a mile. At this time they are occasionally beset by numerous woodpeckers, bluejays, tohees, wrens and sparrows who, with much wrangling and chattering, banquet themselves far beyond their usual bedtime. When darkness has compelled them to desist bats continue the pursuit for another hour. So intent are these creatures upon the chase that it is not a difficult feat to strike them down, and in a woodpecker so secured twenty-six termites were found while a bat had captured thirty-three.

Despite these ravages a large number of insects escape and may be seen flying from one tree to another in search of a suitable spot for the establishment of a colony. Several times I have seen them dashing at door knobs and nail holes in houses and against discolored spots on trees and logs in search of a place where decay has begun. Succeeding in this they frequently shed their wings, though with some this act is accomplished almost at the moment of alighting, while with others it may be postponed until after their excavations have reached a considerable size. Curving the abdomen until it rests across the wings of one side they move backward and usually sidewise, thus bringing the tip of the wings against some construction and causing them to buckle and break off along the line of weakness at their base. The wings are never gnawed off.

It appears that in most cases a female selects the site for the nest and during this process apparently pays very little attention to the one or more males that move about near her. On her falls most of the work of excavation, at least during the first few days, though occasionally when she comes to the outside after a prolonged period of work one of her companions may excavate for a time. In some cases one male appears to be more attentive than the others which often go off on short trips to investigate the cracks and crannies in the neighborhood and may ultimately disappear altogether. In other cases they are driven off by one male and again it sometimes happens that when the burrow becomes a sufficient size to accommodate two, a male takes up a position with the queen and prevents, by fierce lunges, the entry of another. In no case apparently does the female exercise a choice in this first stage of colonization.

Some males are seemingly of a milder disposition than usual, for occasionally one finds two of them in company with one female and, judging by the size of the colony, they have lived together in harmony for more than a year. At other times two females may be associated with one male; and in a few cases I have found two, three and even six pairs living in company. This happens more frequently in captivity, but in any case it apparently disturbs the reproductive process, for the number of accompanying offspring is unusually small. Where royal forms are introduced from other nests a fight almost invariably ensues, and is terminated only with the death of one of the contestants.

It has often been noticed that the antennæ of royal pairs are invariably mutilated. They are, however, intact during the nuptial flight and for three or four days after the entrance into their cell. On several occasions about this time I have seen one individual repeatedly pass one of its antennæ through its mandibles at intervals of perhaps half a minute. Each time it would apparently bite off a small portion and instantly start backward about half its length after the operation. It would then assume a stiff statuesque position with its head rotated to one side or walk with wobbling uncoördinated gait for a little distance before repeating the process. At other times one individual while cleaning the other would gnaw off the tip of the latter's antennæ upon which these same movements would result. The one so mutilated would soon perform the same operation upon the other and so on in alternation, the final result being the reduction within a few hours of one, often both antennæ, to more or less of a stump-like condition. This operation does not prevent the royal pair from making their way about nor from finding their way back to their cell if removed from it for a distance of several inches. In fact the mutilation in no visible way affects their existence, and it is difficult to imagine of what service it is.

Upwards of a week later than this — that is almost a fortnight after swarming — I have on several occasions seen the royal pair of *Termopsis* in coitu. With their bodies closely appressed end to end in a straight line they remain from one to ten minutes in contact, after which they separate without any external indications that the process had taken place. Several times from a few days to many months later than this I have witnessed the same process in various colonies. With one pair kept in captivity this occurred at least a dozen times during eleven months and probably happened more frequently, though in a fairly high temperature (from 20–24° C.), such as is developed by placing them in close proximity to a lamp, this appears to be a much more frequent occurrence than in their normal cool moist habitat.

The swarming of *Calotermes* takes place in January and is quite similar to that of *Termopsis* as is the pairing process. Owing to the fact that I have had but one colony at a time new colonies were not established, but the males and females of the same community not only paired as though more distantly related, but mated and laid eggs. These were non-fertile, however, and soon grew white and disintegrated. *Calotermes* is rather difficult to keep in captivity, but some of these royal pairs lived for nearly five months and assumed almost exactly the size and appearance of the true royal progenitors of the original colony (Fig. 1) which were present in each of the two nests.

Termes lucifugus swarms at different times between the months of October and April. I have watched it more than a score of times and can confirm Grassi's observations in all essential particulars. It usually takes place about 11 A. M. and lasts nearly two hours. During its occurrence a few birds make their appearance and they, together with lizards (*Sceloporus*) and *Formica rufa*, reduce the size of the swarm somewhat though not seriously.

After their short flight the wings are almost immediately shed and the greater number of individuals pair, the male following immediately behind the female. According to Grassi (Blanford's translation, *t. c.*), "the one in front attempts to run away from the other, which pursues it and palpitates the extremity of its abdomen, and sometimes the sides as well. . . . I believe that the meaning of these supposed amorous displays is entirely different from that usually assigned to them, and that the pursuer wishes to solicit the dejecta from the one pursued."

FIG. 1.

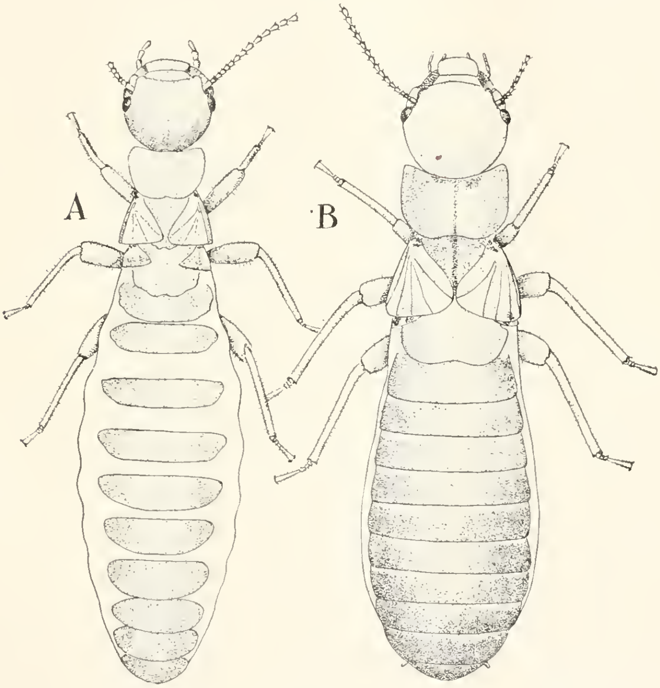

FIG. 1. Primary queens of (*A*) *Termes lucifugus*, eight months old, × 14; (*B*) *Calotermes castaneus*, probably not less than four years old, × 9.

This may be the correct explanation, but there are some facts that throw much doubt upon it. In the first place no food is taken by the perfect insect from the time of its last molt until the work of excavation has commenced and dissections show the alimentary canal to be almost empty. Secondly when one termite solicits dejecta from another it scarcely ever pursues it for more than a centimeter unless there are signs that the scybalum is forthcoming, and even then the pursuit is only for a short distance. But here in the case of *Termes* one perfect insect often pursues the other for many meters, and I have noticed in the case of several which fell in some large cement basins that they kept it up for an entire afternoon and evening. Furthermore, if the individual behind becomes separated from the one in front the latter almost immediately stops and stands transfixed with the abdomen curved upward until it once more feels the palpitation of the antennæ of the one behind. The whole process suggests that some secretion located at the posterior extremity of the body attracts the one behind until the other finds a suitable spot for a nest.

Within a short time after mating the female burrows into the soil where this is soft ; otherwise she traverses some crevice and disappears from sight followed by the male. In Italy, according to Grassi, their subsequent careers are without result. Nevertheless, this investigator was able to obtain the commencement of a colony and Perris[1] notes that in the Landes royal pairs accompanied by eggs are of frequent occurrence under chips. In California this species is very difficult to keep, owing to its inability to withstand even slight changes in the amount of moisture in the nest. It thrives best in large jars filled with chips and earth, but even in this habitat it rarely lives over three or five months. Royal pairs, when placed in these surroundings, after swarming, disappear almost immediately and the following day may be seen next to the bottom of the jar a foot or so from the surface, excavating hollows in the wood and neighboring soil. Five weeks later I have found small clumps of eggs which in one colony hatched, but the young as well as the parents soon died.

Under natural conditions it is almost impossible to find these royal pairs, owing to their habit of burrowing in the soil, but I have secured four colonies after upwards of a year of watching. Three of these occupied excavations in some old fence posts almost completely buried in an unused gravel pit near San Jose. Placed in essentially the same conditions one and a half to two and a half feet beneath the surface they contained seven, nine and thirteen eggs and six, three and eight young respectively. The other colony was placed under and in a plank buried about a foot beneath the soil near Stanford University and contained the true royal pair, seven eggs, one soldier and twenty-seven workers and young of different sizes. From these few examples I am strongly of the opinion that the establishment of new colonies by means of true royal pairs is not only a normal process in California but very probably one of comparatively frequent occurrence.

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[1] Ann. Soc. ent. France (5), VI., 1876, p. 201.

As previously noted the true royal forms of all three species here considered, abstain from any form of nourishment from the time of their last molt until the process of excavation has begun, and no special supply of food is carried from the paternal roof in order to enable them to exist until the time arrives when they may be cared for by their offspring. On the other hand everything at this time is apparently sacrificed to lightness of body, resulting in a wider dispersal with less exhaustion than would result with forms loaded with fully developed reproductive organs or food either in or out of the body.

A very short time after excavation has commenced the abdomen of both male and female increases rapidly in size. That of the latter is slightly larger and more globular, but in both the enlargement is due to nutriment taken into the alimentary canal and to the developing reproductive elements. Within a month both have attained to nearly their maximum size which, as the figures show, is far short of that of some of the tropical forms. At no time do they require to be fed, and their size does not prevent their active locomotion from place to place.

Rate of Increase, etc.—As previously mentioned eggs are deposited in the newly made nests of *Termes lucifugus* within five weeks after pairing, but owing to the fact that these and the young are shifted about in galleries often away from the light, the observations I have made concerning the rate of egg-laying and the time required for hatching are very incomplete. This is also the case with *Calotermes*. *Termopsis*, on the other hand, is much more hardy and lends itself readily to observation and this, connected with their abundance, has placed me in possession of considerable data. They mate readily in captivity, and upwards of

500 true royal pairs now in my possession are in excellent condition after more than a year of captivity. They are also very abundant in recently killed trees, and it is an easy matter to use these colonies for checking up results. Stripping off the bark of such trees from three to nine months after the swarming period I have found scores, in fact hundreds, of small colonies accompanied by the royal pair.

Up to the present time no record has been made of the occurrence of a true royal pair within the United States. Messrs. Banks and Chittenden have kindly called my attention to a brief note by Hagen[1] to the effect that the late Mr. H. G. Hubbard found the queens of *Termes flavipes* in Florida. In this note Hagen remarks that the "females are undeveloped, being wingless, but sexually mature," which shows that they were substitute and not true royal forms. The royalty of such small species as *Termes* and *Calotermes* might easily escape detection, but as *Termopsis* is relatively huge and also abundant and widely distributed throughout the Pacific slope it is remarkable that they have not hitherto been discovered.

For *Termopsis* the date of egg-laying and the consequent development of a colony differs widely according to circumstances. When the habitat is unusually moist a royal pair may remain together for a year without producing offspring, and, on the other hand, under favorable conditions upwards of 75 young may be developed within this period of time. Usually a fortnight elapses from the time of pairing until the first egg is laid; and from one to six are deposited daily until the total number amounts from 15 to 30. Egg-laying now ceases and the parents give their time to enlarging the nest or to caring for the eggs which are objects of careful attention, being kept scrupulously clean and frequently shifted from place to place in the nest.

At the present time my observations are not sufficiently complete to justify an extended account of the rate of growth of the developing young, the number of molts they undergo and the origin of the various castes. With the primary royal pair these phenomena are continually subject to a gradual change. For example, early in the life of the colony a large-headed larva

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[1] *American Naturalist*, Vol. 10, 1876, page 62.

arises as a result of what appears to be the third molt, and after three succeeding ecdyses it becomes a perfectly formed soldier. The latter , in long-established communities, is about 18 mm. in length and possesses 22 antennal segments, while the one first developed is but 10 mm. in length and has 18 or 19 segments. Upwards of three months later another immature soldier appears larger than the first and evidently formed as a result of more than three molts. More than three are also apparently required to give it the adult length of 12 mm. and usually 19 or 20 antennal segments. The new arrival, after the mandibles have become sufficiently chitinized, usually asserts its superiority by putting an end to the first. Other soldiers appear upon the scene from time to time, generally a little larger and occasionally with more antennal joints. Finally an equilibrium is established in this respect, but not until the colony has been established for a year at least.

The first workers also undergo their final molt at an early stage and, as with the soldier, those which appear later assume larger proportions until, finally, at the end of nearly two years, a limit is reached with some of the members which have attained a length of approximately 14 mm.

So far as I have seen nymphs do not occur until after the first year, in other words the first swarm of perfect insects leaves the nest at the end of the second year. They also are relatively small.

The life of all these first inhabitants is comparatively short. The king and queen early draft them into service, but when their place may be taken by other and more powerful individuals they disappear. In some cases they are undoubtedly dispatched, but it is by no means certain that this is always the case. After the colony is fully established soldiers and workers live at least two years and probably longer, though of this I cannot be positive. Regarding the king and queen it is certain that they live together for at least two years in the case of *Termopsis*. In three of the largest two-year-old colonies in my possession there are in the neighborhood of 200 individuals, while in some colonies headed by the royal pair I have found more than 1,000. From such nests many perfect insects have swarmed, and it is reasonable to believe that they have existed for five years at least. There is not the slightest evidence that the kings do not live as long as the queens. After the death of the royal pair from natural or other causes the members of the orphaned colony (to use Grassi's expressive term) develop a number of substitute royal forms. The individuals chosen for this purpose are usually the larvæ of perfect insects in which the wing buds are barely visible. By some unknown process, possibly by a peculiar method of feeding or some change in their diet, the external larval characters are retained (the larvæ undergoing no subsequent molts), while the reproductive organs are stimulated to active growth culminating in full functional activity. Very shortly, often within a month after the death of the primary pair, these substitute forms may be detected, owing to their faint straw color, which rapidly grows to a very much darker tint during another month.

If only one of the royal pair be destroyed usually only one substitute form is developed, but when both perish from ten to forty substitutes appear, according to the size of the colony. In two nests carefully examined I found six substitute males and eleven females in one, and nine males and thirteen females in the other. As might be expected, these do not mate permanently, and one male may pair with three or four queens during the course of an hour.

The substitute individuals are fed and cleaned almost entirely by the workers, which also assume the care of the young. Accordingly the royal task consists almost altogether of egg-laying and is performed with comparative rapidity. Some of the larger queens (Fig. 2) lay continuously from seven to a dozen eggs in twenty-four hours and where several are associated together the colony rapidly assumes large proportions. In the extensive nest of *Termopsis* mentioned on page 48 there were over thirty substitute individuals which were congregated beneath a small strip of bark where the sun shone brightly, and with them was a clump of eggs, attended by many workers, that I carefully estimated at not less than 8,000.

In colonies where either the king or queen persists the substitute royal individual is invariably, so far as I know, an immature perfect insect, but where both have perished the substitute royalty may sometimes contain a worker or a nymph or even a soldier capable of laying eggs. Such monstrous forms are not infrequent in large orphaned nests, but never apparently in colonies headed by the true royal pair. One may also find winged soldiers, soldiers with mandibles of varying size, and, as just mentioned, soldiers with wing buds, the straw-color characteristic of substitute forms and with functional reproductive organs. These last-named insects are comparatively rare. I have had but three in my possession. All of them laid eggs in captivity and in one case I followed the development for a long period of time, but the young and the nymphs and workers into which they became transformed, appeared in all respects perfectly normal.

FIG. 2.

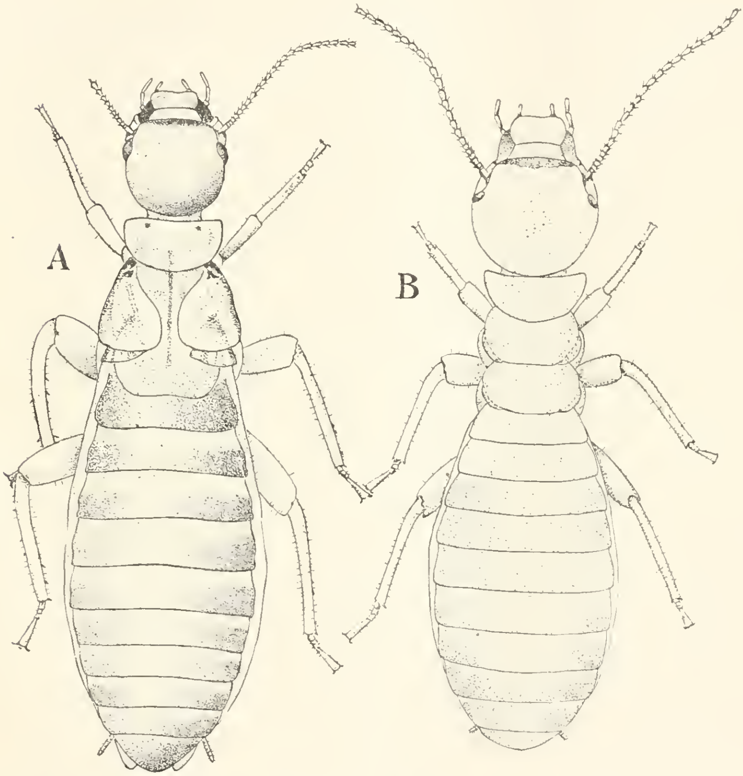

FIG. 2. *A*, primary queen of *Termopsis angusticollis*, at least four years old, × 6; *B*, complemental queen of same species, × 7.

Food, etc. — The food of termites consists almost wholly of wood gnawed directly from the wall of the burrow or solicited from some other individual. The primary royal pair appear to live almost altogether on food which they scrape off for themselves, When the young are hatched they are fed for some time on material regurgitated by the parents, but after a molt or two proctodeal food is added and finally constitutes almost the entire food supply procured from another individual.

After having fed a colony on oak or redwood (*Sequoia*) which produces dark-colored dejecta, and then changing the diet to white pine, it is easy to see that the greater part of the excrement, voided in the form of white pellets, never again enters the digestive tract of another individual. Apparently this is chiefly owing to its dryness. Some specimens, probably all at different times, appear to have the power of retaining a certain amount of moisture in the excreta. These are eagerly sought out by their companions which palpitate the abdomen of the one solicited with their antennæ and clasp its tip between their mandibles. If the stimulus be too early applied the one sought after runs away ; otherwise it passes out the pellet which is greedily devoured. Occasionally one may feed itself in this way. This proctodeal food supply constitutes almost the sole diet of the substitute royalty, whose dejecta, like that of the soldiers, is never again devoured.

It appears to be a general belief that the soldiers, on account of the huge size of their mandibles, are able only with the greatest difficulty to solicit proctodeal food, and are accordingly in a chronic state of hunger. Sick or disabled individuals are greedily eaten by them, and at various times perfectly healthy forms are dispatched and devoured. Grassi supports this belief by some examples, but at the same time he notes that *Calotermes flavicollis* also solicits food in the usual way. With all the Californian species the soldiers are almost invariably at peace with their fellows. Moribund and possibly supernumerary forms are dispatched, but this is rarely done by the soldiers, who stand guard most of the time at some of the more exposed parts of the nest. At other times they move quietly among the workers and nymphs, and, with heads rotated to one side, solicit food with very little difficulty. After watching many colonies for more than a year I have yet to see a soldier subsisting on any other kind of food. On one occasion I saw a soldier of *Calotermes* trying industriously to gnaw off part of the abdomen of a disabled nymph and at another time watched one biting a splinter of wood, but these efforts were of short duration and without success.

FIG. 3.

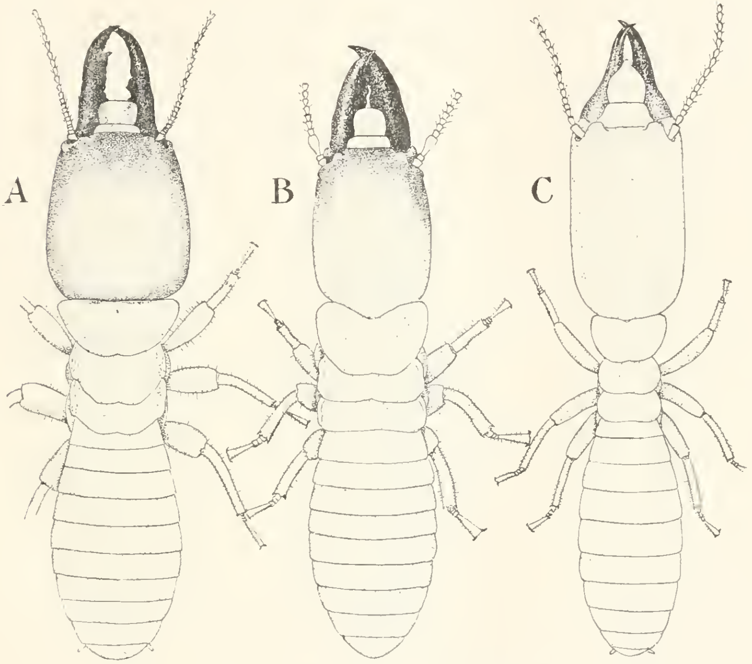

FIG. 3. Termite soldiers. *A*, *Termopsis angusticollis*, × 5; *B*, *Calotermes castaneus*, × 9; *C*, *Termes lucifugus*, × 12.

On several occasions, while transferring colonies from their nests into jars, I have seen the soldiers of all three species rush frantically about and impale one of their fellows on their mandibles, but after quiet had been restored such acts were never repeated and it is safe to conclude that they form no part of the normal life of a colony.

In the presence of sufficient moisture the discarded rejectamenta are often used in the construction of barricades or for filling old beetle burrows; otherwise they are cast outside the nest.

Some species are said to cement the particles together by means of saliva or material regurgitated from the anterior part of the alimentary canal, while others employ the method of *Termopsis* and *Calotermes.* These, carrying a pellet in their mandibles, travel to some spot where building is required, and, feeling about with their antennæ, locate a breach in the wall. Facing about they then deposit a drop of liquid proctodeal material into which the excrement is quickly pressed with many waggings of the head, and immediately rush after another pellet which is likewise stuck fast. By this time the cement has set and the worker or nymph departs into the crowd, where it rests and is groomed by its fellows. In their natural surroundings the amount of building done by these insects is relatively small, but colonies kept in glass cases and in the presence of sufficient moisture, erect low but extensive roofs, elevated on many pillars, that serve to protect them from the light and other injurious agents.

It is now thoroughly well established that the soldier and worker in the termite colony are not the result of the arrested development of the reproductive organs. It has also been shown that they are not restricted to either sex. And it is almost equally certain that their differentiation is not to be traced back to the newly hatched young. The latter, when they first appear, are exactly alike in form and color, though they may exhibit slight differences in size, and the characteristics of the different castes develop at varying times after the first molt. If then neither arrested development, nor sex nor heredity are directly responsible for the production of soldiers and workers, what is the agent immediately concerned? Grassi is of the opinion that it is the food. Owing to its character or amount or both, the royal pair or the colony are able to transform the larvæ into soldiers, workers or perfect insects. Such a belief gains some support from allied phenomena among the social hymenoptera, but at the present time very little definite knowledge exists regarding this subject for the termites. For months I have fed a large number of termite colonies of all ages, with or without royal pairs, on various kinds and amounts of food — proctodeal food dissected from workers or in other cases from royal forms, stomodeal food from the same sources, sawdust to which different nutritious ingredients had been added—but in spite of all I cannot feel perfectly sure that I have influenced in any unusual way the growth of a single individual. However my experiments along this line are far from being completed and it appears best to hold the account of this phase of the subject for a future time.

Stanford University, Cal.,
October, 1902.

A PRELIMINARY NOTE ON THE OCCURRENCE OF A FILARIA IN THE CROW.

J. H. ELLIOTT, M.B.,

Late George Brown Memorial Scholar, University of Toronto, Physician-in-Charge, Muskoka Cottage Sanatorium, Gravenhurst, Ont.

The discovery of a number of species of filariæ in birds in southern Nigeria in 1900, when studying the hæmamœbæ, as a member of the Liverpool Malaria Expedition to Nigeria, suggested the question whether these parasites were to be found in birds in temperate climates.

Few opportunities offered in the course of my duties, but a few bluejays and crows were secured during the summer of 1901. The bluejays were examined with negative results. Of eleven crows three were found to have embryo filariæ in the blood, all of one species. In two of the three the red corpuscles were infected with *Halteridium.*

The search for the parent forms of the filariæ was not successful, and owing to the migration of the crows, further study had to be deferred until the following summer.

This year I have been able to secure only four crows. One of these fortunately was infected with the same filaria found in 1901. After careful dissection and teasing of the tissues the parent forms were found in the coats of the pulmonary artery. In appearance they resemble the human form *F. Bancrofti.*

The embryos vary greatly in quantity in the blood stream of the different birds. In the fresh preparation their movements are rapid, and they progress readily in either direction. By ringing with vaseline they will keep active for several days. The average length of a number measured in the fresh preparation was 187.96 microns. In permanent cover-glass smears, dried, fixed in alcohol, and then stained, the length varies somewhat, averaging 173.1 microns.

What appears to be the head end is blunt or abruptly rounded, the anterior two thirds being of uniform diameter. The posterior third tapers gradually until it reaches about one half the width of the anterior end, when it is abruptly rounded. In the fresh condition no sheath could be made out nor does it show in preparations stained with fuchsin, but with hæmatein a sheath is well defined extending however only slightly beyond either the head or tail.

In stained preparations .05 of the anterior end is clear; at .37 from anterior end and extending to .4 there is in most specimens a paler spot in which there are few nuclei; at .6 from the head end, and extending to .7 there is a clear spot in all the specimens in which no nuclei are seen, and little or no stain is taken. This spot and its fixed position is characteristic of this embryo, and clearly distinguishes it from any filaria which has been previously described. At this spot in the stained specimen the diameter is 5.8 microns, as compared with 4.7 microns at the blunt end, and 3.4 microns at the tapered end.

I hope very shortly to give a detailed description of this new filaria, and the embryos found in the circulating blood.

GRAVENHURST, ONT.,
October 16, 1902.

THE ORIGIN AND DEVELOPMENT OF THE GASTRIC GLANDS OF DESMOGNATHUS, AMBLYSTOMA AND PIG.

MARY J. ROSS,

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Introduction.

A precursory study of the glands of the pig's stomach successfully demonstrated at the very beginning of this investigation the difficulties attendant upon a true understanding of the real origin of the stomach glands from the study of such a complex form. I am glad, here, to acknowledge my indebtedness to Dr. B. F. Kingsbury, who suggested the form *Amblystoma*, and to Mr. C. H. Boxmeyer, who suggested, and assisted me in procuring the material for the study of *Desmognathus*. It is to the study of this latter form I ascribe any success I may have had in this investigation.

Many investigators have been interested in the origin and function of the different cells, as parietal and central, in the glands of the stomach, but few have studied the development of the glands for itself alone. Thus only can one account for the lack of accurate information on this subject. The present paper goes, perhaps, to the opposite extreme, in being concerned solely with the origin of the glands from the earliest recognizable stages, until it can be clearly demonstrated that true glands are formed. In *Desmognathus* this has been comparatively simple, in *Ambly-*

stoma, less so, on account of the great number of yolk spherules obscuring all details; while in the pig the complex structure of the glands has rendered this especially difficult.

TECHNIQUE.

As both *Amblystoma* and *Desmognathus* abound in Ithaca a complete series of each was obtained. The material was fixed every other day and accurate measurements taken, so that a full series, differing less than ½ mm. in total length between each, was secured. Fixation was accomplished in various ways: Kleinenberg's picro-sulphuric (distilled water, 100 c.c.; sulphuric acid, 2 c.c.; picric acid to saturation, 1 part; distilled water, 3 parts), Flemming's chrome-acetic (water, 19 parts; 1 per cent. osmic acid, 16 parts; glacial acetic acid, 2 parts; 10 per cent. chromic acid, 3 parts), picro-nitro-sublimate (sat. aq. sol. of picric acid, 500 c.c.; water, 300 c.c.; nitric acid, 24 c.c.; mercuric chlorid to saturation), and Perenyi's fluid (10 per cent. aq. sol. of nitric acid, 4 parts; 95 per cent. alcohol, 3 parts; ½ per cent. aq. sol. of chromic acid, 3 parts) all gave fairly good results. But Carnoy's, modified (glacial acetic acid, 30 c.c.; absolute alcohol, 30 c.c.; chloroform, 30 c.c.; nitric acid, 3 c.c.; mercuric chlorid to saturation), fixing ten to sixty minutes, washing in 67 per cent. alcohol, then in 82 per cent. alcohol and iodin, and Gilson's, modified (glacial acetic acid, 5 c.c.; nitric acid, 5 or 10 c.c.; 95 per cent. alcohol, 100 c.c.; sat. aq. mercuric chlorid, 400 c.c.), fixing three fourths to twelve hours and washing as in Carnoy's, gave the most satisfactory results.

Many different stains were used, as methylene blue and eosin, eosin and orange G, carmine-picro-nigrosin, thionin, safranin, iron hematoxylin, but for staining sections on the slide chloral hematoxylin with eosin as a counter stain gave the best results. It was also found very advantageous to stain material fixed in Gilson's or Carnoy's in toto. Eosin, restaining with hematoxylin and eosin, or borax-carmine (Grenacher), restaining with hematoxylin, proved satisfactory. Staining in toto with Delafield's hematoxylin and counter-staining in toto with a saturated alcoholic solution of eosin yielded excellent results, as there is very little danger here of overstaining. (Small pieces were stained

24–36 hrs. in Delafield's hematoxylin, Del. hem., 1 part, + distilled water, 4 parts, then washed in distilled water 10–12 hrs., or until the water is no longer colored by the hematoxylin. They were then stained in toto with eosin, sat. sol. in 95 per cent. alcohol, washing out with absolute alcohol.)

For the earlier stages, on account of the great amount of yolk present, double imbedding with collodion and paraffin was employed. Thinner sections could be obtained in this way, with less loss of yolk material. In thickness the sections varied from two to ten micra.

The material used in the study of the stomach of the pig was placed at my disposal by the Department of Histology and Embryology. It is a great pleasure to express my appreciation to the members of this Department for the interest shown during the investigation.

This material was fixed in five per cent. formalin, mercuric chlorid, alcohol, picric alcohol and Zenker's fluid. In spite of the tendency of the epithelium to shrink away from the mucosa the formalin material proved the best for study. Staining in toto with alcoholic eosin, restaining with hematoxylin and eosin, or staining in toto with Delafield's hematoxylin and alcoholic eosin was employed with good success. Other stains as mentioned above for *Desmognathus* and *Amblystoma* were also used.

All figures are drawn with an Abbé camera lucida. Unless otherwise stated drawings are made with Leitz lenses and the ordinary tube length. Objective 1/16, oil immersion.

Origin of the Gastric Glands.

Desmognathus.

Notwithstanding the general tendency of to-day to explain the development of the so-called higher forms by comparison with the simpler or lower, nothing has been done, as yet, along this line, for the gastric glands. The embryologies are singularly free from references as to the origin of glands, save in the higher forms, and even here there is a dearth of helpful literature.

To review, briefly, the facts, as known in the Batrachian forms so far investigated, will aid to simplify the presentation of this subject. The œsophageal glands of *Urodela* have received more or less consideration as the question of the homology of these glands, with one another, and with those of higher forms has for a long time aroused much interest. But, leaving this interesting problem entirely out of the discussion, there are described in the stomach of some of the *Urodela* two kinds of glands, the anterior and posterior oxyntic glands of Langley ('81). Bensley ('00), for *Amblystoma*, and Carlier ('98), for *Triton*, have given excellent descriptions of these glands. In the adult form the glands consist of a duct, composed of cells resembling the surface epithelium, a neck, composed of clear mucous cells, and a body, made up of granular cells containing large nuclei. The glands of the cardiac, fundic and pyloric regions vary in complexity but all resemble, to a greater or less extent, the type form just described. These anterior oxyntic, or cardiac glands, may or may not be transition forms between the true gastric glands and

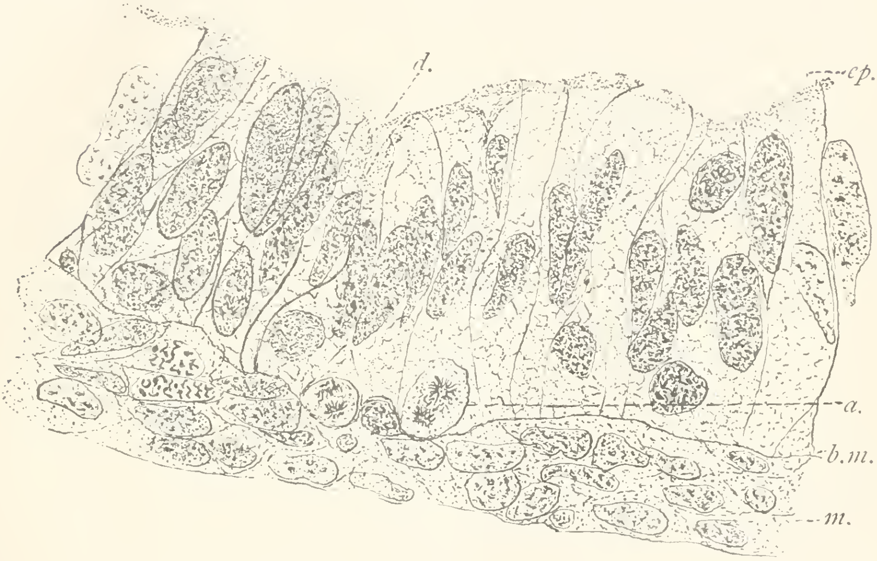


FIG. 1. Transection through the fundus of the stomach of a larval *Desmognathus*, 11.5 mm. Fixed in Gilson's, modified. *a.*, gland anlage, "ovoid cell," just about to divide; *b.m.*, basement membrane; *d.*, insinking at the surface of the epithelium to form the future excretory duct; *ep.*, epithelial lining of the stomach, showing one of the cells in process of division; *m.*, mesodermic tissue forming the muscular layers about the stomach. This also shows mitoses. Obj. $\frac{1}{16}$, Oc. 1.

the large œsophageal glands. But, whatever they may be as to homology, all of the glands of the stomach are alike in the formation of their early stages.

The early stages in the formation of the gastric glands in *Desmognathus* are easy to discern, as in this form there is comparatively little yolk present to obscure the cell outlines. The first traces of the future glands are found in a larva of 11 mm. total length. The cells are at first apparently many layers thick, but on careful inspection it may be seen that they are arranged in an orderly manner like simple columnar epithelium as shown in Figs. 1 and 2. The nuclei, thus arranged at different levels, give to the epithelium a stratified appearance, which is somewhat misleading at first sight. In the same figures insinkings or slight indentations on the free surface correspond to the cells whose nuclei lie deep in from the surface (Figs. 1 and 2, *d*). At the base of the surface epithelium, resting almost upon the basement membrane, are large round cells, with round or oval nuclei (Figs. 1, 2, *a*). These cells and nuclei are larger, clearer, and more

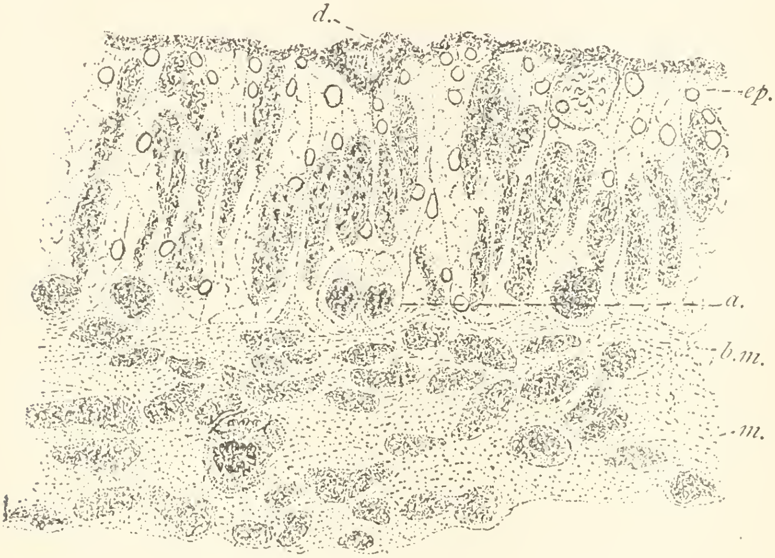


FIG. 2. Cross-section through the cardiac region of the stomach of a larva 11.5 mm. long. Gilson's. Letters as in Fig. 1. This shows a round cell dividing in two. Obj. $\frac{1}{16}$, Oc. 2.

finely granular, resembling the "ovoid" cells described by Sewall ('82) in the sheep, and the "ersatzzellen" described by Toldt ('80) in the cat. In sections stained with hematoxylin and eosin the nuclei of these cells take a deep blue, and the cells a deep red color. This enables one to trace the changes in these cells in *Desmognathus* with comparative ease. Even in larvæ less than 11 mm. in length these round cells may be observed. They appear with the differentiation of the stomach from the general entoderm. These cells then divide, as shown in Figs. 1, 2 and 3, forming groups of two, three and four cells. In Fig. 1 are two of these cells in a state of division. All of the cells of the epithelial and subepithelial layers appear equally capable of multiplication but the gland anlages at this stages show marked

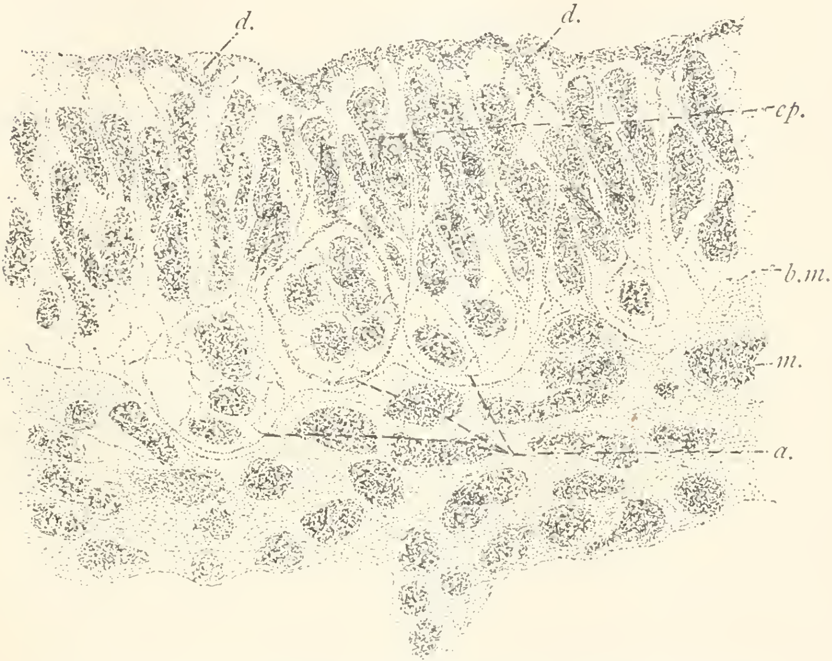
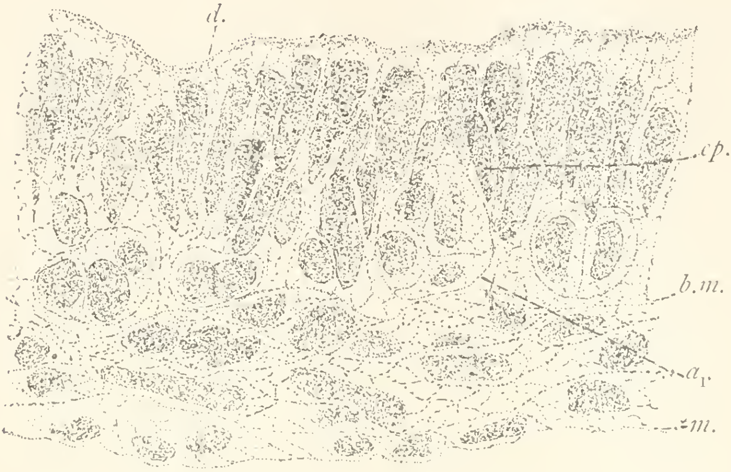


FIG. 3. Cross-section through the fundus region, where the pancreas is attached to the stomach; *a.*, gland fundaments in various stages of development, forming groups of several cells. By their growth they are displacing laterally the cells of the surface epithelium, *ep.*, making the insinkings more marked, *d.* Other letters as in Fig. 1. Obj. $\frac{1}{16}$, Oc. 2.

mitoses. Thus by repeated division these small rounded masses of cells increase in size. It does not appear probable that these



FIGS. 4–7. The following four sections form a series representing one gland throughout its whole extent. Transections (10 μ) through fundus of a larva of 13 mm. Fixed in Gilson's. Letters as in Fig. 1. Obj. $\frac{1}{16}$, Oc. 1.

FIG. 4. a_1, end view of gland, showing some of the epithelial cells of the surface projecting around the end of the gland.

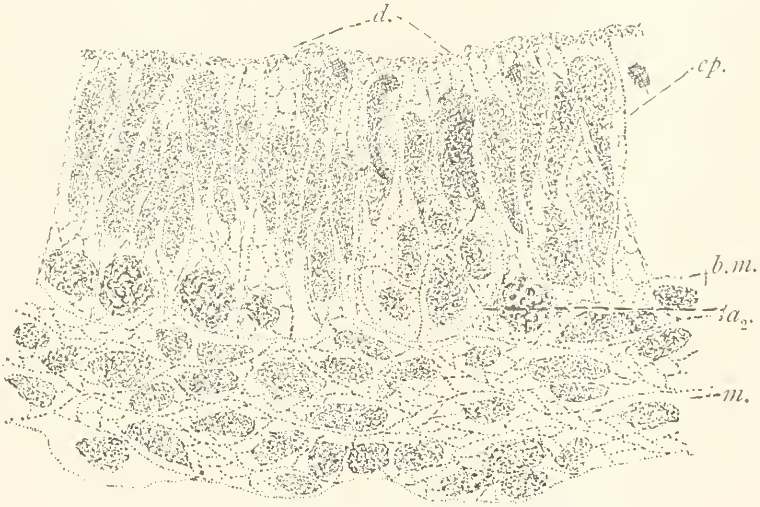


FIG. 5. a_2, transection through the center of the gland.

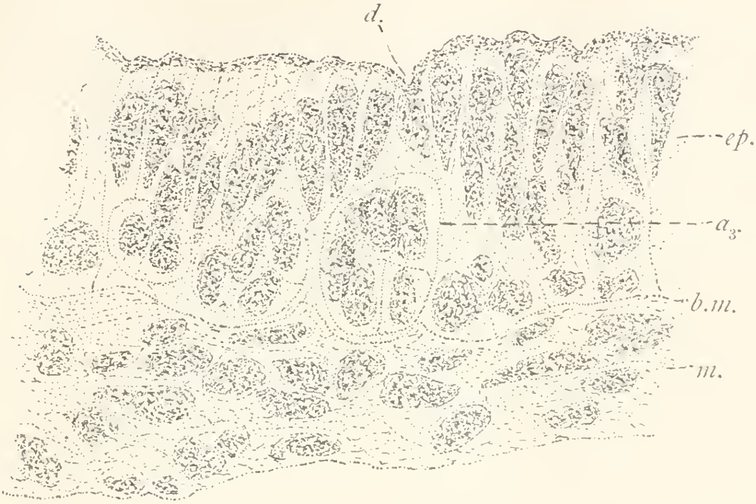


FIG. 6. a_3, same as Fig. 5. No lumen shows as yet.

small round cells at the base of the epithelium coalesce to form a gland, as no trace of such aggregation could be found, but that each cell gives rise to an individual gland. While this process is

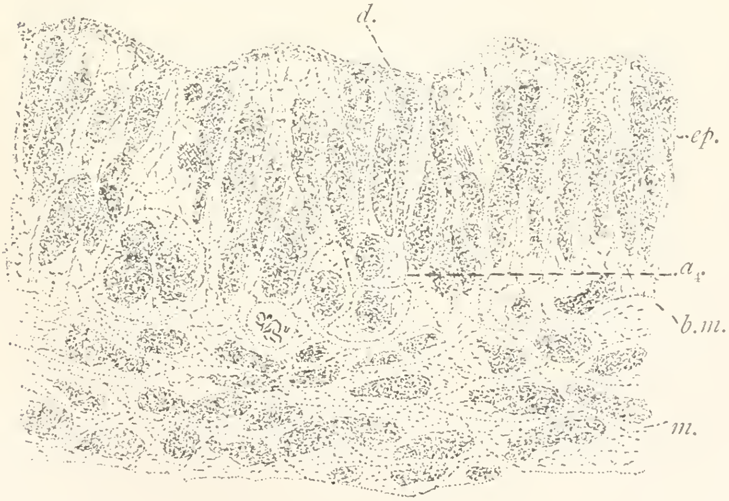


FIG. 7. a_4, same as Fig. 4.

going on the insinkings at the surface (Figs. 3, 10, *d*) continue to deepen, forming, in *Desmognathus*, narrow tubes. The gland fundament, by rapidly increasing in size, is forcing its way, not down into the mesodermic layer, though there is a slight burrowing in this direction (Figs. 1, 3, *a*), but toward the lumen of the stomach. The epithelial cells of the surface are being pushed out of their original position, and arranged around the gland fundament as a central axis (Figs. 3, 4, *cp*). As the free surfaces of the cells are thus withdrawn from facing the cavity of the stomach to form the lumen of the gland, or, speaking more accurately, to form the excretory duct, the epithelial cells adjoining the gland proper, or fundus, become rounder and appear in a more active condition than the rest of the epithelium. This is shown in Figs.

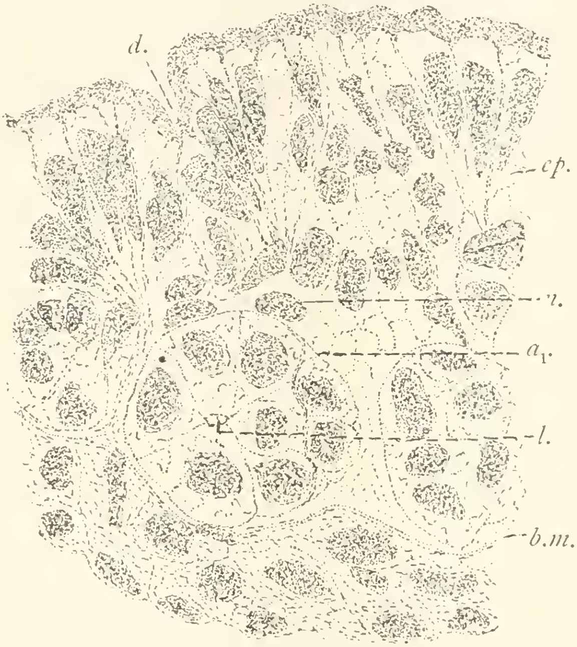


FIG. 8–11 represent a series of sections through a gland of the pyloric region of the stomach of a 16 mm. larva. Fixed in Gilson's. Letters as in Fig. 1. Obj. $\frac{1}{16}$, Oc. 1.

FIG. 8. a_1, transection through a gland fundament showing the lumen; *d.*, the insinking at the surface is more marked than in earlier stages; *n.*, cells which later take part in forming the neck.

4, 5, 6 and 7 of a larval *Desmognathus* 13 mm. long. The gland fundament here extends laterally throughout at least four sections (40 micra), or, perhaps, six as it is extremely difficult to trace out all the individual cells of such a series. As yet there is no lumen apparent in the gland (Figs. 1–7). A section through the center of the gland is shown in Fig. 6. This shows the insinking (*d*) at the surface, and the arrangement of the nuclei of the surface epithelium around the gland as an axis (*a*). Fig. 4, a section nearer the edge of the gland, shows the arrangement of the nuclei of the cells around one end of the gland (*a*). This same series (Figs. 4–7) also shows another interesting fact, which indeed holds true for all the animals studied. While the stomach is being differentiated from the entoderm all the above processes are taking place at the same time, so that in the same series are found gland fundaments in different stages of development. So,

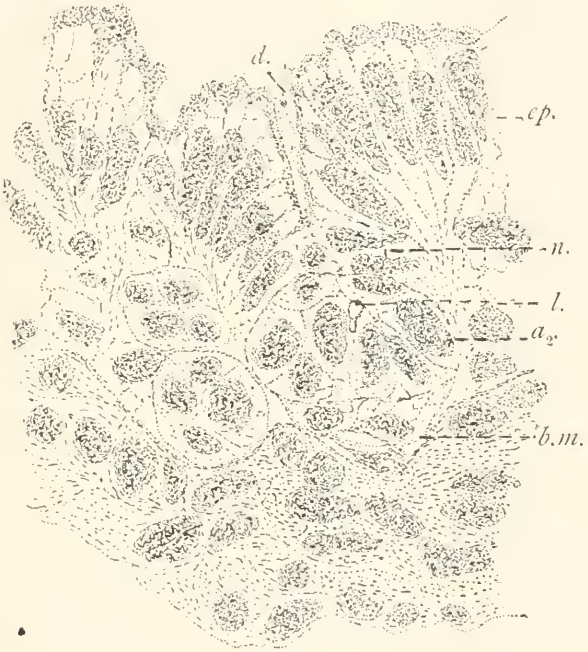


FIG. 9. Same as Fig. 8. a_2, the cells of the gland fundament are arranging themselves about the gland lumen in a way to indicate the approaching union of the duct and fundus at *n.* (the neck cells); *ep.*, the epithelial cells show the lateral displacement, which gives rise to the duct, to a marked extent.

as in Figs. 4, 9, 12, the more advanced and earlier stages are often seen in the same section. In no stage, however, were the two extremes, *i. e.*, a completed gland and a beginning one, found.

In a larva of 16 mm. the stomach is entirely formed, but as yet no glands open on the surface. Figs. 8–11 show glands further developed than those just described. By the increase in size of the gland and the multiplication and rearrangement of the cells lining the stomach, the excretory duct is being formed (Fig. 9, *d*). Before the connection between the fundus of the gland and the excretory duct is established, a lumen is formed in the gland fundament (Figs. 8, 9, *l*). This appears to be caused by a rearrangement of the cells and not by a cell disintegration. The connection between the duct and fundus, forming the neck of the gland, is also brought about by a rearrangement of the cells and not by a cell disintegration. In Figs. 12–17 some of the glands

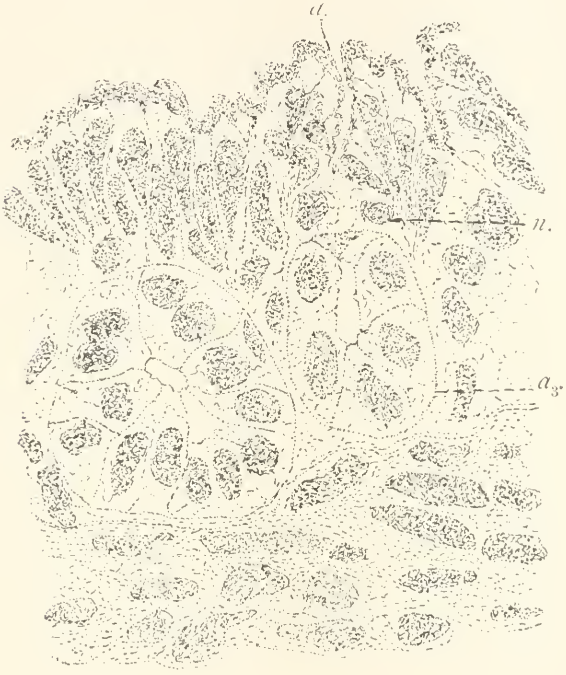


Fig. 10. Same as Fig. 8.

open to the cavity of the stomach. The neck cells, connecting the excretory duct and fundus, appear in a strongly active condition (Fig. 15, *n*). This maturing of the gland occurs just at the time of hatching, so that when the *Desmognathus* is hatched some of the glands are completely developed (Fig. 13, *n*) while others are still incomplete (Fig. 13, a_2). Several days after hatching all the glands open to the surface of the stomach. When

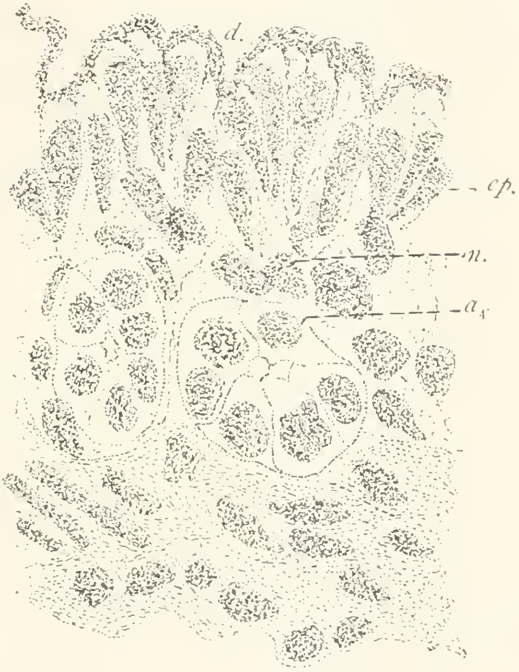
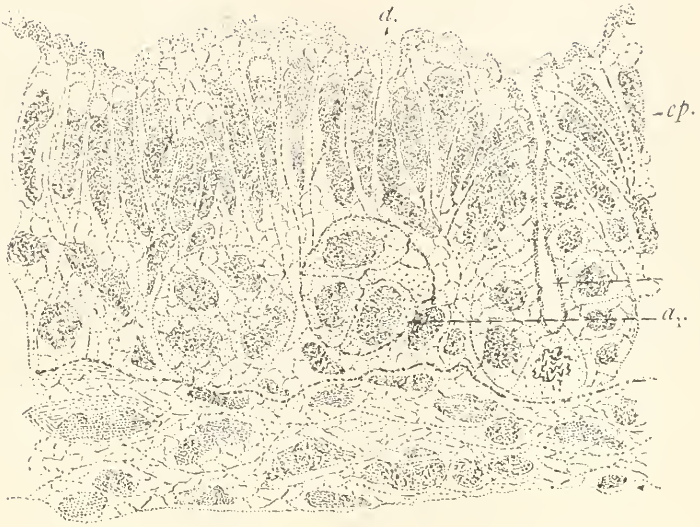


FIG. 11. Same as Fig. 8.

this has taken place no more glands are formed in the way just described, but further increase is by a subdivision of the fundus of the gland, in the same manner as later described for the pig.

Amblystoma.

In the *Amblystoma* the gland anlages are not readily demonstrated as the cells of the epithelium lining the stomach are completely filled with yolk. Staining the sections on the slide with hematoxyln, eosin and picro-fuchsin aided materially in over-



FIGS. 12–17 represent a series through a gland, showing the union of the duct and the fundus. This is taken from the central region of the stomach of a larva *Desmognathus* at the time of hatching. Glands in all stages of development, from the one-celled anlage up to a completely formed gland, are shown thoughout the sections. Fixed in Gilson's. Obj. $\frac{1}{16}$, Oc. 1.

FIG. 12. a_1, transection showing merely the outside cells of the gland.

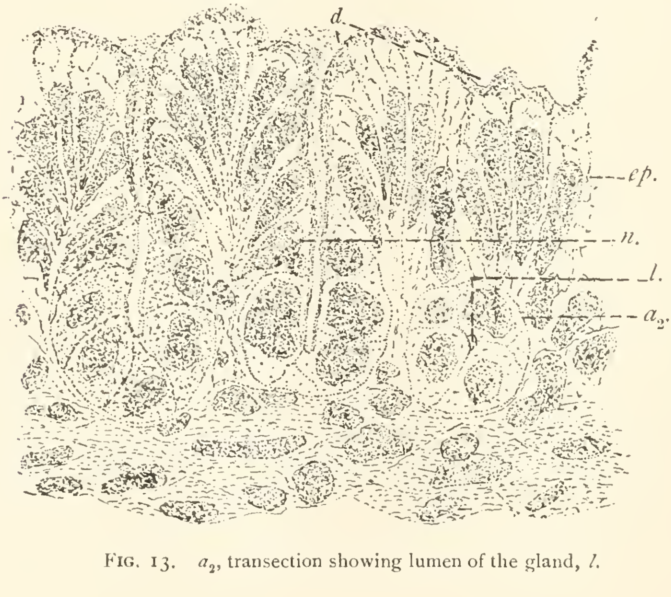


FIG. 13. a_2, transection showing lumen of the gland, *l*.

coming the difficulties caused by the large amount of yolk. The nuclei took a deep blue color, the yolk spherules became a transparent orange yellow, while the round cells, so characteristic of this stage in the *Desmognathus*, stained a deep red. In a larva 11 mm. total length, although the caudal portion of the stomach is yet undifferentiated, these large, granular, round cells are found at the base of the epithelium (Fig. 18, *a*). Fig. 20, *a*, shows one already divided, while in Fig. 20 are gland fundaments in several stages of development. Some extend laterally for about 30 micra. Here, as in *Desmognathus*, the epithelium appears to be made up of several layers of cells, the deeply staining nuclei (Figs. 19 and 20) being arranged at different levels. The cells lining the cavity of the stomach seem to be fewer in number on account of their great distention with yolk, consequently do not present the appearance of alternate layers to such a marked degree as was shown for *Desmognathus*. This orderly arrange-

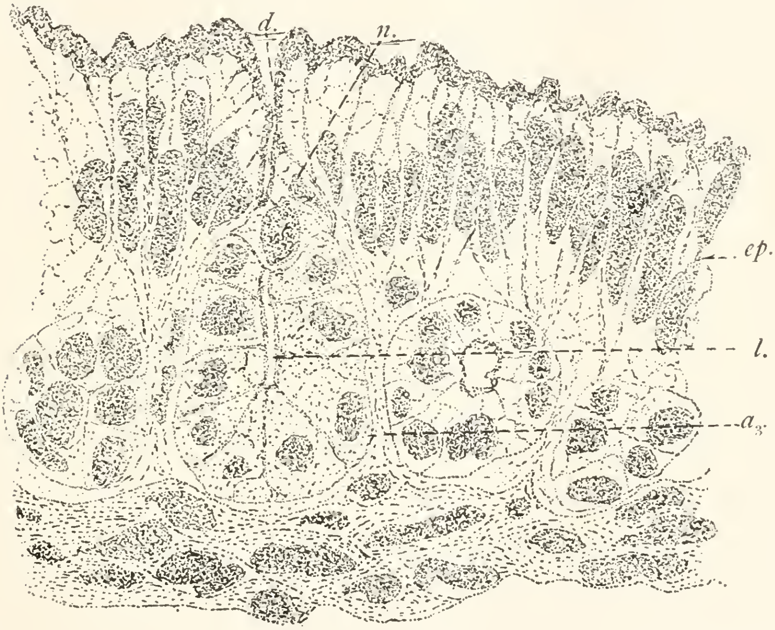


FIG. 14. a_3, gland showing lumen, *l.*; *d.*, deep indentation with a rearrangement of cells of the epithelium to form the duct.

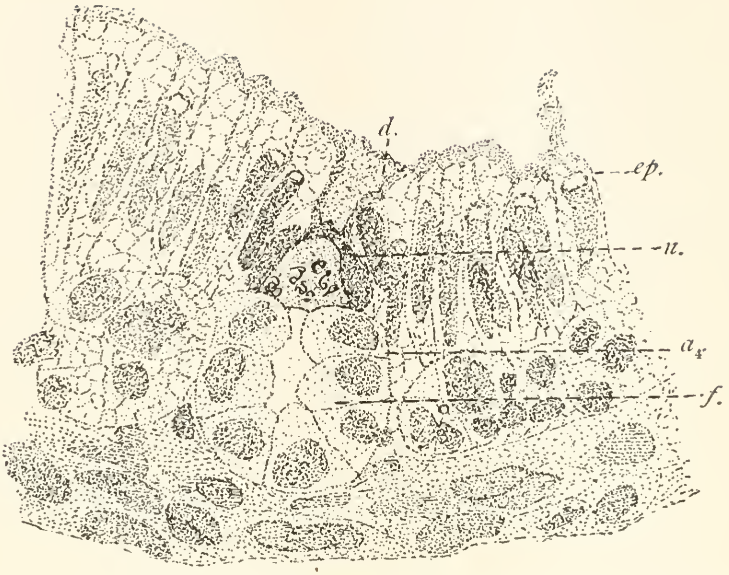


FIG. 15. a_4, gland showing the large clear cells, so characteristic of the earlier stages. The cells at the upper part are in process of division, showing the union of the duct, *d.*, with the fundus, *f.*, to form the neck *n.*

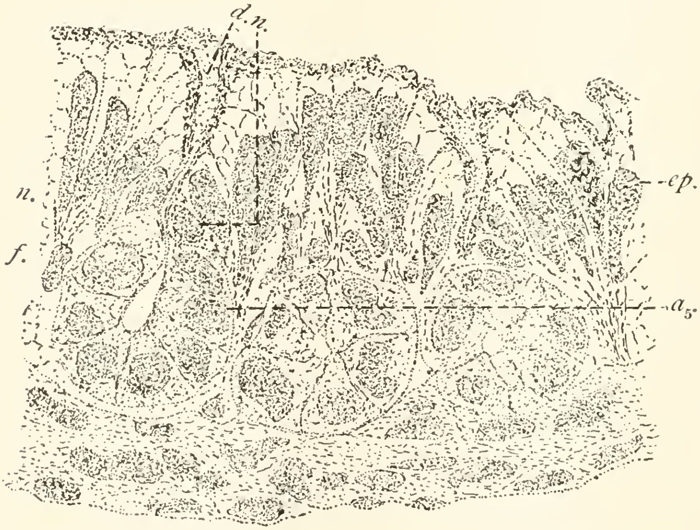


FIG. 16. a_5, section showing the opening of the gland to the surface.

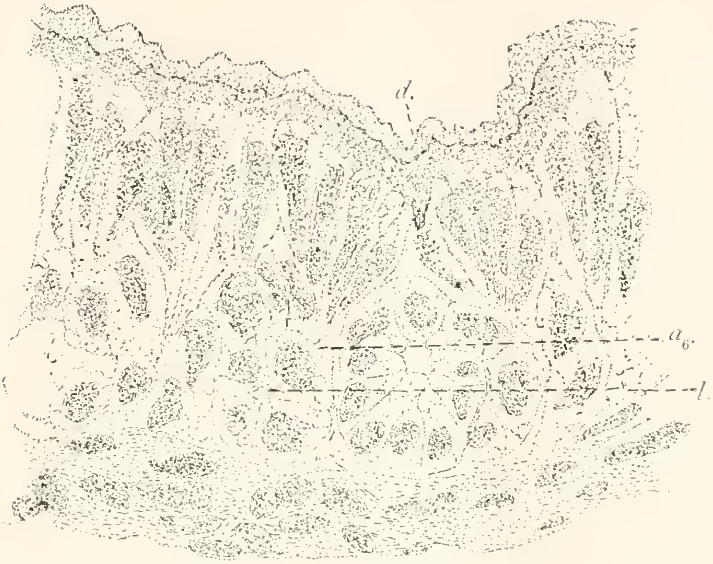


FIG. 17. a_6, same as Fig. 12, a_1.

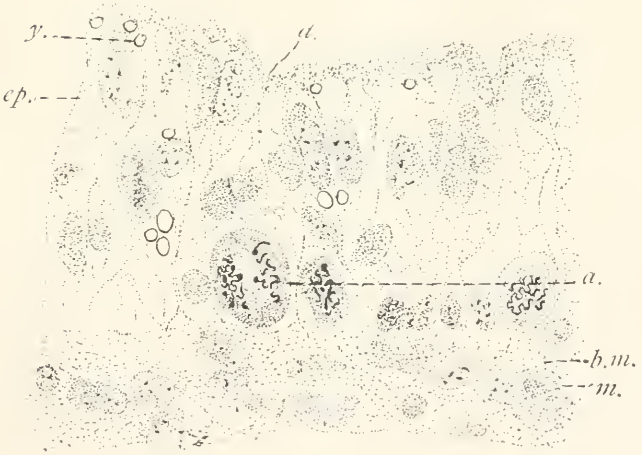


FIG. 18. Cross-section through the cardiac region of larval *Amblystoma* of 11 mm. total length. Gilson's, modified. *a.*, "ovoid cell," or gland anlage, in process of division; *b.m.*, basement membrane; *d.*, depression at the surface to form the future excretory duct; *ep.*, epithelium of the surface; *m.*, mesodermic tissue forming the muscular layers; *y.*, yolk spherules which obscure the cell outlines. Obj. 7, Oc. 2.

ment of nuclei at different levels also corresponds to the insinking at the surface, shown in all of the series of *Amblystoma* studied (Figs. 18, 19, 20, *d*). As the gland fundaments increase in size by cell division the yolk spherules become absorbed; the epithelium lining the stomach becomes narrower and lower, until in a larva of 12 mm. (Fig. 20) a stage comparable to Fig. 3 of *Desmognathus* is obtained. The yolk disappears from the glandular epithelium more rapidly than from the surface epithelium. Thus the origin of the fundus of the gland is more readily traced than the development of the excretory duct. Figs. 21 and 22 in a larva 16 mm. in length show slight insinkings or indentations at the surface. In the same figures the gland fundaments have progressed further in development than in the corresponding stage in *Desmognathus*. The fundus of the gland extends throughout several sections and the cells have already become arranged around a central lumen (Figs. 21 and 22, *l*). The cells which are to form the excretory duct and neck, although arranged around the gland as a central axis, do not present a marked opening. Instead of a narrow oval slit, as in a corresponding stage of *Desmognathus*, there is formed a shallow pit. From this

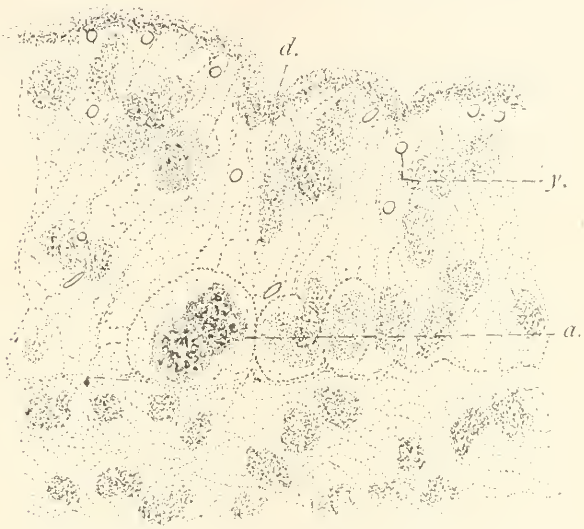


FIG. 19. Same as Fig. 18. The primary round cells now divided to form two. Obj. 7, Oc. 1.

stage there takes place a rapid disappearance of yolk, a rapid multiplication of cells and a correspondingly rapid insinking of the surface epithelium to form the excretory duct. At the time of hatching there is, as before described for *Desmognathus*, a union of the gland fundus with the excretory duct, the cells showing marked mitoses at this point (Fig. 23, *n*).

There are several points in which the *Amblystoma* appears to differ from the *Desmognathus*. The cells of the surface epithelium are fewer in number, being distended with yolk. The excretory duct is shorter, the glands opening on the surface of the stomach by very short necks. As the yolk is absorbed the columnar epithelium lining the stomach becomes lower; the gland fundaments increase more rapidly than the surface epithelium, as the

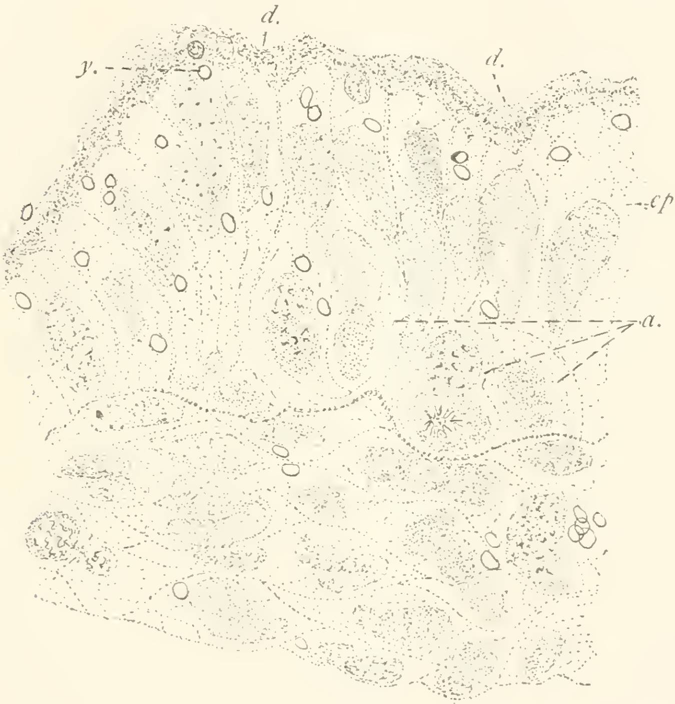


FIG. 20. Same as Fig. 18. Larva 13 mm. *a.*, groups of cells, two, three and four, by increasing in size, are pushing the epithelial cells aside. Thus the indentation, *d*, is being deepened. The yolk is also being absorbed, making the epithelium, *ep.*, narrower or lower. Obj. $\frac{1}{16}$, Oc. 2.

yolk disappears from them first, hence there is little space left for the formation of excretory ducts. The glands, then, are shorter and more flask-shaped than in the *Desmognathus*, though here too are found simple tubular glands.

Bensley ('00) describes the gastric glands of *Amblystoma* as "tubular downgrowths" of entoderm. "The anterior glands," he says, "are of a flask like shape and have a distinct lumen." "The posterior glands are simple tubes, composed of a simple layer of yolk-filled cells surrounding a cleft like lumen." From the glands described and figured by Bensley it seems probable that the "cleft like lumen," with the nuclei arranged as if sur-

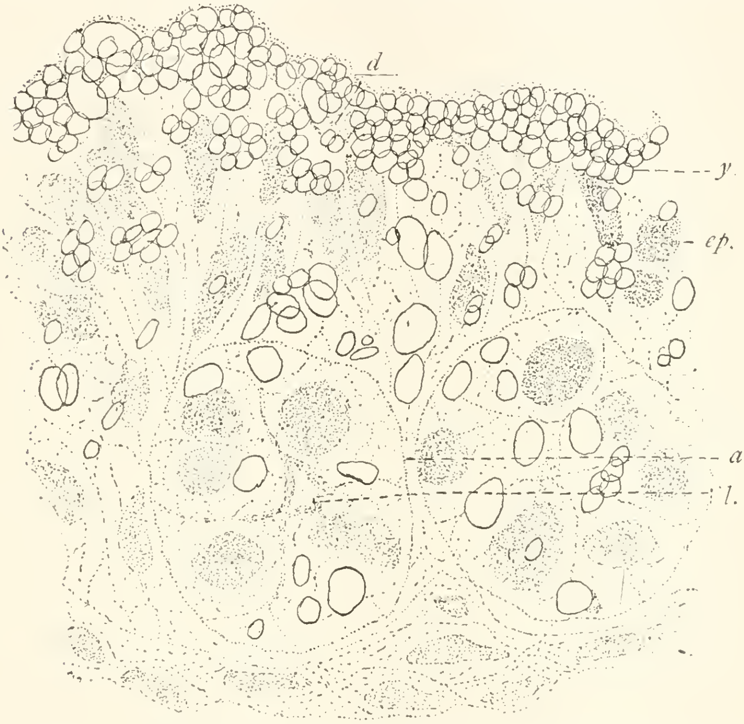


FIG. 21. *a.*, the gland fundament has increased in size very rapidly and now has a central lumen; *d.*, the duct has not developed to a corresponding extent, being as yet only a shallow pit; *ep.*, the epithelial cells are becoming radially arranged around the gland fundament as an axis; *l.*, central lumen; *y.*, the yolk is still present in the epithelial cells of the surface, though it has almost disappeared from the gland cells. Obj. $\frac{1}{16}$, Oc. 2.

rounding it, represents merely the excretory duct and, perhaps, the neck, while the fundus of the gland is to be sought for in the aggregation of cells he has portrayed at the base of his so-called gastric gland. Or this may be but one of the longitudinal ridges and depressions, cut transversely, which abound in the œsophagus but are less numerous in the stomach proper, though at an early stage they are seen here also.

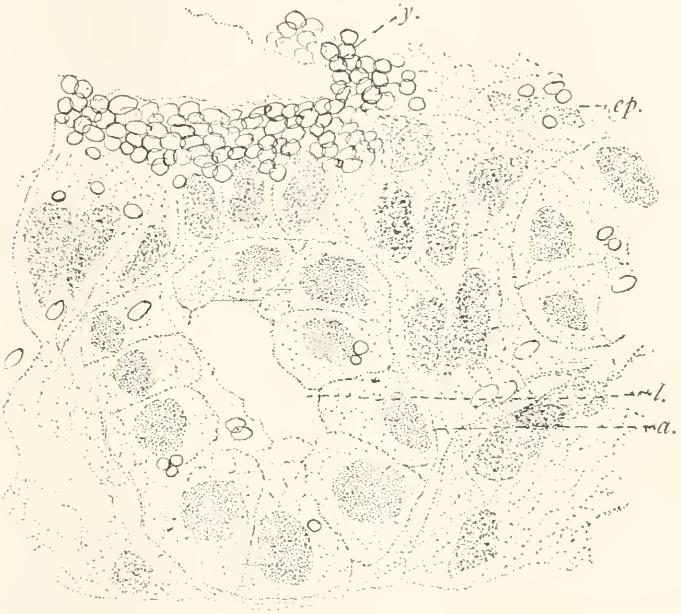


FIG. 22. *a.* shows a gland fundus almost fully formed while as yet the epithelial cells, *ep.*, are full of yolk, *y.*, and the duct, *d.*, is still a shallow pit; *l.*, lumen. The gland reaches almost to the free surface of the stomach. Obj. $\frac{1}{16}$, Oc. 2.

Pig.

The general course of development of the earlier stages of the gastric glands of the pig takes place in exactly the same manner as in the simpler forms already described. As early as in an embryo pig of 2 cm. total length, round, granular cells appear, differing from the remainder of the epithelial cells by their structural appearance and also by their characteristic staining reaction when stained with hematoxylin and eosin. The cells lining the stomach are so closely packed together that they appear to be composed of several layers. Sections cut at 2 micra show that the epithelium is simple columnar; all the cells which reach the surface also rest upon the basement membrane, the "pyramidal cells" of Toldt ('80). In this respect the earlier stages in the pig also resemble those found in *Desmognathus* and *Amblystoma* (Figs. 3, 19, 24). These round cells, appearing at the base of the epithelium, rest upon the basement membrane (Fig. 24, *a*). They then divide, as described before for *Desmognathus* and

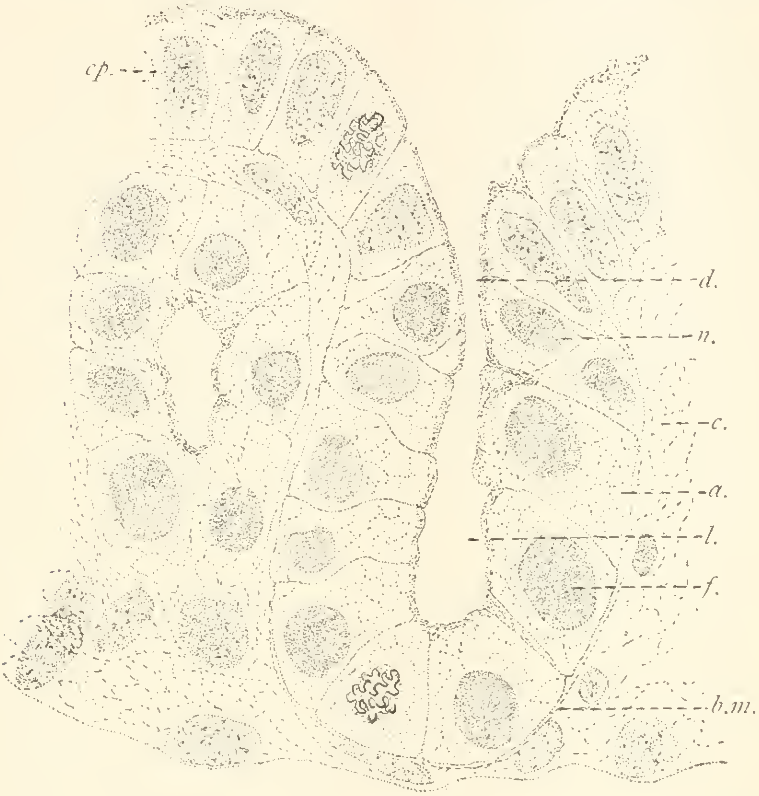


FIG. 23. Cross-section through the stomach of an *Amblystoma* of 16 mm. length. The yolk has all been absorbed. This shows the gland in the adult condition. Sections of larvæ of this size also show stages comparable to Figs. 13–18 of *Desmognathus*. Fixed in Flemming's fluid. *bm.*, basement membrane; *c.*, connective tissue; *d.*, duct, which is very short in *Amblystoma* due to its late development; *ep.*, epithelial cells; *f.*, fundus; *l.*, lumen; *m.*, muscular layer; *n.*, neck of gland. Obj. $\frac{1}{16}$, Oc. 2.

Amblystoma, forming groups of two, three and four cells (Figs. 24, 25, *a*). While in both the other cases the epithelium was relatively thick, here it is very thin. For, as the gland anlages divide and increase in size, the epithelial cells gradually lose their stratified appearance, and become arranged in a single layer. The nuclei, instead of being arranged at different levels (Fig. 24, *a*), assume their place in the center of the cells (Figs. 28, 29, *cp*). Before this process is completed the gland fundaments cause a displacement of the surface cells, as in *Desmognathus* (Figs. 25, 26, *d*). The cells of the gland are arranged around a central lumen, small to be sure, but still a lumen (Fig. 26, *l*). There is also a slight indentation at the surface, as the cells are forced out of their original position (Fig. 24, *d*). Thus in an embryo of 6.2 cm. length (Fig. 25, *d*) there is a stage similar to that shown in *Desmognathus* and *Amblystoma* (Figs. 3, 20, *d*). The insinking resembles that of *Amblystoma* rather than *Desmognathus*, as it is merely a shallow pit. This deepens as the gland fundaments increase in size and the connection between the fundus and the duct is then established (Fig. 27, *n*). Immediately after the

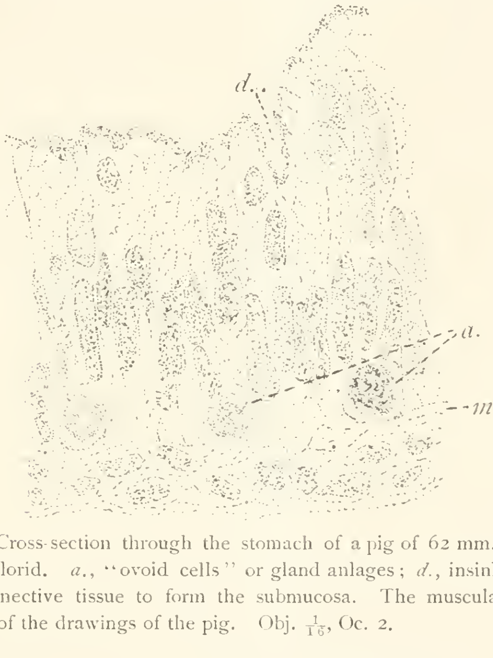


FIG. 24. Cross-section through the stomach of a pig of 62 mm. length. Fixed in mercuric chlorid. *a.*, "ovoid cells" or gland anlages; *d.*, insinking to form the duct; *m.*, connective tissue to form the submucosa. The muscular layers are not shown in any of the drawings of the pig. Obj. $\frac{1}{16}$, Oc. 2.

opening of these shallow pits or indentations into the lumen of the gland, and even in some cases before that has occurred, the cells of the surface epithelium and mesoderm begin to multiply rapidly, projecting into the cavity of the stomach. These first outgrowths are, as Sewall ('79) describes them, in the form of short ridges, not of villi, as described by Brand ('78) and Negrini ('86). By continued outgrowths along these depressions and ridges the ridges intersect, resulting in a fine network. This is clearly seen with the naked eye in stomachs taken from pigs 14

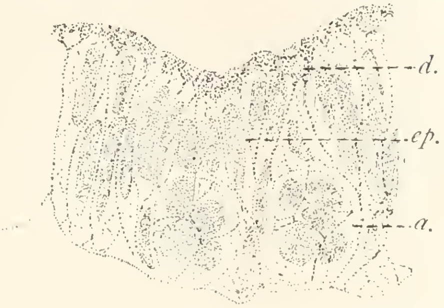


FIG. 25. Same as Fig. 24. *a.*, the original round cells have now divided, forming groups of several cells; *d.*, the indentations at the surface have deepened till they resemble those of *Amblystoma*; *ep.*, the epithelial cells are becoming radially arranged around the gland fundaments.

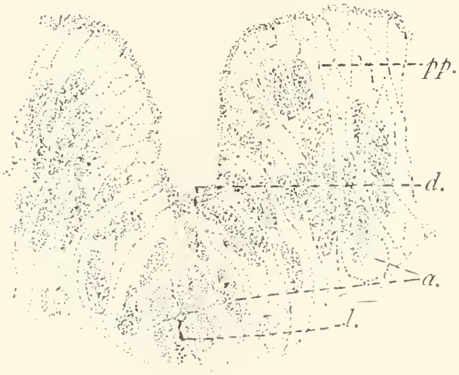


FIG. 26. Same as Fig. 24. Here the epithelium is increasing in size, pushed out also by increase in growth of the mesoderm to form the primary outgrowths or processes, *pp.* The glands are nearer the surface and the pits for the ducts, *d.*, are thus being deepened; *l.*, small central lumen.

cm. in length. These projections are termed "glandular processes" by Sewall because he considers them to be the first evidence of glandular formation, but it seems more probable that their primary object is to increase the epithelial surface of the stomach. The glands, which are little more than pits or depressions, are seen at the tops of the ridges, at the sides of the ridges and in the depressions between the ridges. The glands are but little deeper than the original insinkings of the cells of the surface, but the fundus is readily distinguished as the cells are round, clear, staining intensely red with eosin, while the cells of the duct retain the appearance characteristic of surface epithelium

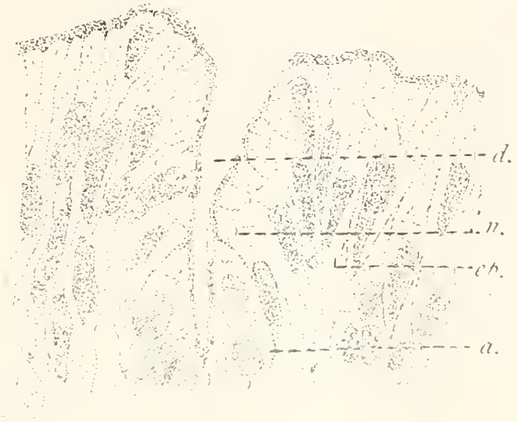


FIG. 27. Same as Fig. 24. *a.*, the gland fundament has here united with the duct, *d.*; *n.*, neck cells which are clearer and rounder than those of the surface epithelium; *ep.*, epithelial cells of the surface, not a part of the duct.

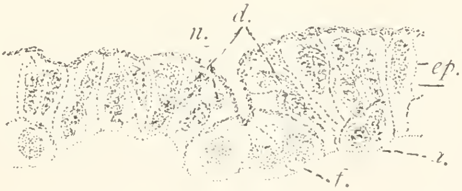


FIG. 28. Represents a section taken from the cardiac region. Similar sections are to be found in the fundus and also in the pyloric region; *f.*, fundus; *n.*, *d.*, neck and duct; *ep.*, epithelium. This may have been partially caused by artificial distension of the stomach. Formalin. Obj. $\frac{1}{16}$, Oc. 2.

(Figs. 30, 31, 32, *f*, *d*). The duct cells are a little rounder and more granular than the rest of the stomach epithelium, hence they might more properly be termed neck cells (Figs. 27, 28, 32, *d*) as they correspond to the neck cells found in *Desmognathus*

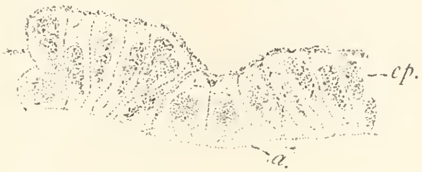


FIG. 29 represents a section from the fundus of the same stomach as in Fig. 28. These two figures are taken from an earlier stage than Figs. 30 and 31, before the primary processes have begun to develop.

(Fig. 14, *n*). In this case the greater part of the future excretory duct still lies on the surface as surface epithelium. This is probably the true explanation, for the cells around the lumen of the glandular pits multiply and project out in all directions into the cavity of the stomach, forming secondary ridges. These secondary growths (Fig. 32, *sp*) appear on the sides and crests of the

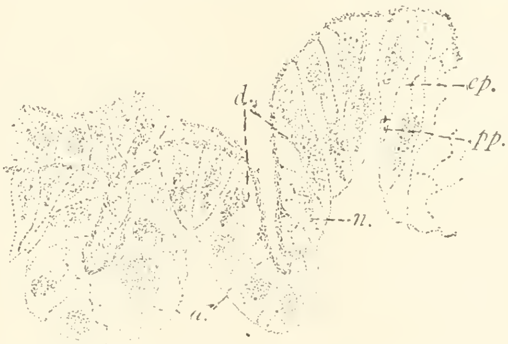


FIG. 30. Section through the pyloric region. Here is shown the same condition as in Fig. 32. Letters as in Fig. 28. Formalin. Obj. $\frac{1}{16}$, Oc. 2.

ridges already formed and in the depressions or hollows between them. The connective tissue also takes part in the formation of these secondary processes, projecting upwards as a central core, as shown in Fig. 32, *c*. At first these projections do little more than deepen the shallow pits, before described. As development proceeds further these processes become approximated (Fig. 32, *d*) and, in this way, the excretory ducts are formed. The fundus of the gland, composed of deeply staining granular cells, remains near the basement membrane. In a later stage the fundus also elongates.

To recapitulate briefly: the fundus of the gland is formed from clear round cells lying at the base of the epithelium; the neck is formed from the epithelial cells which take part in uniting these to the surface epithelium; the excretory ducts are formed secondarily — by outgrowths of the single layer of epithelial cells, covering the processes of the primary outgrowths of the mesoderm, and also by a continued insinking of the surface epithelium.

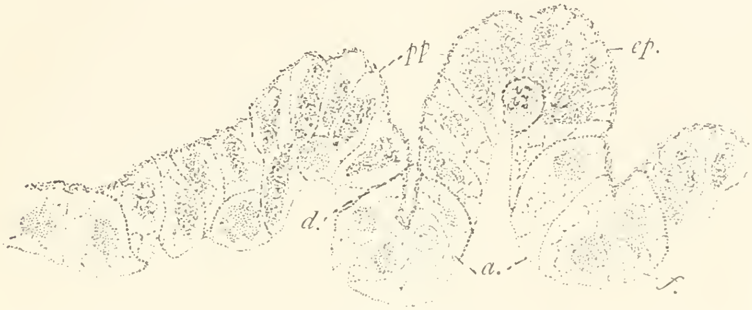


FIG. 31. Transection through the fundus of the stomach of a pig 16.5 cm. long. Same as in Fig. 32. Formalin. The mucosa is pushed out even straighter than in the preceding figure, so that the gland cells open almost on the surface of the stomach.

It is not surprising that to these solid epithelial and mesodermic outgrowths, or projections, should have been ascribed the origin of the glands by Kölliker ('52), Laskowsky ('68), Brand ('78), Negrini ('86) and Salvioli ('90). For, in the pig at least, the round clear cells are very small, while these so-called "gland processes" are so marked and present so striking an appearance that it is little wonder that they should have attracted the most attention, and in them should have been sought the sole explanation of the glandular origin. In 1880 Toldt described, for the cat, solid epithelial masses "Ersatzzellen," as occurring at the base of the epithelium. These, he said, became arranged in a special form, degenerated in the center to form a vesicle or miniature gland. The vesicles then lengthened and divided to form glandular pits. This is the nearest approach to a correct description anywhere found. Salvioli ('90) and Pilliet ('86) consider that the glands are formed by an insinking of the surface epithelium, as a cul-de-sac or vesicle at the base of the crypts or between the "gland processes." Sewall ('79) also described "ovoid cells" lying at the base of these crypts. He said that "the ovoid cells first appear in the deep part of the glands, and in older embryos they may be traced on, assuming more and more their completely developed characters, and becoming more numerous and extending farther up the gland." Instead of this being the case, from one of the glands described by him, many, in reality, are formed, as was described above. It appears probable that the numerous "transitional forms" he finds between the "ovoid" and "embryonic" gland cells, are the neck cells, which occur at the place of union of the gland fundament and the excretory duct. The neck cells would at this stage be more marked and more numerous, relatively, than could be satisfactorily explained on other grounds. Thus, also, can be explained what appeared to him a "wholly unexpected phenom-

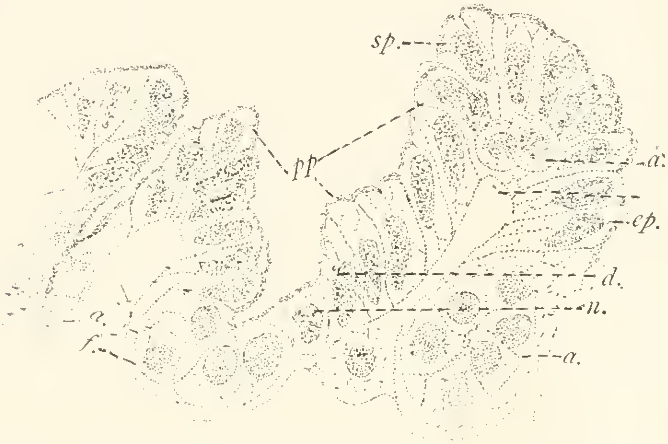


FIG. 32. Section through fundus of stomach of pig. Formalin. *a.*, gland anlage ; *f.*, fundus of gland which has now, by the further growth of the "primary processes," *pp.*, been straightened out so that by looking down on a surface view, it is merely a shallow pit ; *n.*, neck cells ; *d.* later form the duct ; *ep.*, epithelial cells of the surface ; *sp.*, secondary process forming on the crest of a primary process. Obj. $\frac{1}{16}$, Oc. 2.

enon" — the presence of these ovoid cells throughout all the glands of the embryo stomach. These round cells do appear throughout the whole stomach wherever glands are to be formed, at the pyloric, cardiac and fundic regions in all three types examined. They are, as has been stated, the gland anlages, and are to form the functional portion of the glandular epithelium. A pig embryo of 10 cm. shows these round or "ovoid" cells at the base of the gland, resting upon the basement membrane (Fig. 32, *f*). A section through a pig of 14 cm. shows them still further depressed from the surface, appearing more truly gland shaped (Fig. 30, *f*). In this stage the glands are beginning to subdivide. Division takes place at the base of the gland and also along the sides of the fundus of the gland. Cells proliferate and project up into the lumen of the gland supported by connective tissue outgrowths. Several glands may thus come to open into one duct by the formation of these new outgrowths. These, in their turn, are subdivided by similar outgrowths. As all these open at the neck or in the fundus of the gland, compound glands are formed from what were originally simple tubes. This also takes place in the same manner in *Desmognathus* and *Amblystoma*.

In tracing the development of the earlier stages of the glands the whole stomach could be sectioned. In most cases the pancreas was left attached as it also assisted in rendering orientation easier. In pigs of more than 12 cm. total length portions were taken from different parts of the stomach, as cardiac, fundic and pyloric regions. Here, as in the earlier stages, glands were traced from section to section, every precaution being taken not to confuse them with one another, by taking accurate measurements of all the sections.

SUMMARY.

The surface epithelium presents, at first, a stratified appearance, but soon becomes a single layer with nuclei approximately in the center of the cells. The stomach glands are formed from round granular cells lying at the base of the surface epithelium. These round cells divide and multiply, forming around a central lumen, while at the same time causing an insinking of the epithelium of the surface to form the excretory duct, by lateral displacement of the surface cells. These then become united — forming at their junction the neck of the gland. So far all three forms agree. But in the pig the epithelial cells multiply and project into the cavity of the stomach to form ridges, supported by mesodermic outgrowths. On these ridges and in the pits between them are found the glands just described. They are lengthened by a further outgrowth of the epithelial cells about their lumen, also supported by connective tissue cores, and a slight downgrowth of the gland proper into the connective tissue beneath. In this way the excretory ducts are formed secondarily in the pig. These round or "ovoid" cells are not characteristic of any one region, but are found over the entire surface of the stomach in all three cases, wherever glands are to be formed. They are specialized very early, as they may be seen in *Desmognathus* and *Amblystoma* before the whole stomach is yet fully formed.

BIBLIOGRAPHY.

Baginsky, A.
'82 Untersuchungen über den Darmkanal des Menschl. Kindes. Virchow's Archiv, Bd. 89, 1882.

Bensley, R. R.
'00 The œsophageal glands of Urodela. Biological Bulletin, Vol. II., No. 3, Dec., 1900.

Brand, E.
'77 Beiträge zur Entwicklung der Magen und Darmwand. Diss. Würzburg, 1877.

Cade, A.
'01 Les Éléments sécretéurs des glandes gastriques du fond chez les mammifères. Archives d'Anatomie Microscopique, Vol. 4, 1901.

Carlier, E. W.
'99 The Newt's Stomach during Digestion. La Cellule, Tome XVI., 1898.

Eisler, P.
'85 Zur Histologie der Magenschleimhaut. Halle, 1885.

Garel.
'79 Glandes de la muqueuse intestinale et gastrique des Vertébrés. Thesis. Paris, 1879.

Greenwood, M.
'84 Observations on the Gastric Glands of the Pig. Journ. of Physiol., V., 1885.

Griffini, L., und Vassale, G.
'88 Über die Reproduktion der Magenschleimhaut. Beiträge zur Pathol. Anat. u. zur allgem. Pathol., III. Bd., 1888.

Krazowski, V.
'80 Untersuchungen über die Entwicklung des Omasus. Inaug. Diss., Dorpat, 1880.

Langley, J. N.
'81 On the Histology and Physiology of the Pepsin-forming Glands. Phil. Trans. Roy. Soc., Vol. CLXXII., 1881.

Laskowsky.
'68 Über die Entwicklung der Magenwand. Sitzungsber. der wiener Akad., math.-naturw. Klasse, Bd. LVIII., 2 Abt., 1868.

Negrini, F.
'86 Intorno allo svilluppo e struttura della mucosa gastrica del majale. Giorn. di Anat. Fisiol. e Patolog. degli animali, XVIII., 121, Pisa, 1886.

Neuman, E.
'76 Flimmerepithel im Ösophagus Menschlicher Embryonen. Archiv f. mikrosk. Anat., XII., 1876.

Oppel, A.
'96 Vergleichende mikrosk. Anatomie der Wirbeltiere, I., Jena, 1896.

Pilliet, A.
'87 Sur l'evolution des cellules glandulaires de l'estomac chez l'homme et les vertébres. Journal de l'anat. et de la physiol., t. 23, 1887.

Pilliet, A., et Talat, M.
'86 Sur les différents stades évolutifs des cellules de l'estomac cardiaque. Compt. rend. Soc. de Biol. Paris, Tome III., 8 Serie, 1886.

Salvioli.
'90 Quelques observations sur le mode de formation et d'accroissement des glandes de l'estomac. Internat. Monats-schrift für Anat. u. Physiol., Bd. VII.; Archives ital. de biologie, Tome XIV., 1890.

Sewall, H.
'79 The Development and Regeneration of the Gastric Glandular Epithelium during Fœtal Life and after Birth. Journal of Physiology, Vol. I., 1879.

Toldt, C.
'81 Die Entwicklung und Ausbildung der Drüsen des Magens. Sitzungsber. d. K. Akademie d. Wissensch., math.-naturw. Kl., III. Abt., Jahrg., 1880.

Töpfer, K.
'91 Die Morphologie des Magens der Rodentia. Morphol. Jahrbuch, Bd. XVII. 1891.

A PRELIMINARY NOTE ON THE ABSORPTION OF THE HYDRANTHS OF HYDROID POLYPS.

H. F. THACHER.

Loeb has found that when pieces of *Campanularia* are placed in dishes of sea water, the polyps in contact with the glass undergo a transformation and disappear completely into the stem. This process, he states, is "due to contact, and is accomplished by the liquefaction and subsequent withdrawal of the protoplasmic mass." In taking up this subject, at the suggestion of Professor Morgan, my wish was to see whether in this case a study of the histology would support Loeb's theory of the liquefaction of the protoplasm as a result of contact: and also whether the process in *Campanularia* resembles that in other hydroids in which absorption occurs, but is due merely to the change from natural to laboratory conditions. For the latter point, in addition to *Campanularia*, I examined *Eudendrium* and a few cases of *Pennaria*, both of which forms also readily absorb their hydranths.

To see if by chance *Campanularia* would also absorb its hydranths when not in contact, I made a set of experiments, placing the splinters of wood on which the hydroids were growing in dishes, so that, as far as possible, the animals would be in a normal position. Under these conditions I found that the polyps were absorbed as rapidly as when touching the glass. These results show at least that contact is not essential to the production of this phenomenon, and suggest the likelihood that the absorption is due to the same cause in all cases.

The beginning of the degenerative changes are first shown by the appearance of large numbers of spherical granules in the digestive current, and by an increase in its rapidity. Shortly after, the polyp, which is to be absorbed, contracts into its cup, and the tentacles fold closely over it. Gradually the polyp becomes shorter and shorter, and the tentacles pass from the length found during ordinary contraction to a knob-like stage, and later are completely absorbed. Towards the close of this period the hypostome also disappears. At this time the digestive current which has been forced periodically from one end to the other of the hydroid-colony, may, by distending the remains of the polyp, delay absorption for a number of hours. If, however, the pressure of the current is not great, the polyp grows gradually smaller until only a small ball of material is left in the cup, and this is then drawn down into the stalk. The whole process may occur in six hours, or may be prolonged for two days or more.

A study of the prepared material shows that changes begin first in the endoderm cells of the body of the polyp, into the cavity of which are thrown fragments of degenerating endoderm and gland cells. This continues for some time, and is accompanied by the contraction of the supporting lamella, as a result of which the ectoderm changes from a flattened to a columnar form. The cells of the hypostome round up rapidly at a comparatively late stage, and are set free into the digestive cavity; the lamella contracting as before. In the tentacles the endoderm is also in process of degeneration, and later, when a break comes in the lamella at the base of the tentacle, the cells pass into the body cavity. The ectoderm cells in this region are thrown into folds which, seen from the surface, might easily give the effect of being fused, as noted by Loeb; but I have not seen any signs of real fusion — only many cases where the cells of different tentacles are brought into close contact. During this time the lamella of the tentacle breaks, and masses of nettle cells and ectoderm pass through the break into the digestive cavity. The broken ends of the lamella now draw together and form a hollow shell, which is frequently much distended by the pressure of the digestive current on the elastic lamella. Degeneration continues by the slow turning in of ectoderm and endoderm cells, until only a small fraction of the original polyp remains, and this is then drawn through the opening at the base of the cup. There are no signs, either external or internal, of any drawing back of protoplasm to form a part of the stalk previous to the final stage, but at this time the strands of protoplasm connecting the cœnosarc and perisarc at the end of the stalk are broken, and in sections the masses of nettle-forming cells, which usually lie in the ectoderm just below the polyp, can be seen to have moved farther down the stalk.

Examination of *Eudendrium* and of *Pennaria* show that the process is the same as that in *Campanularia*, except in those secondary points to which the structural differences of the hydroids would give rise. There being no cup, the tentacles remain separate during absorption, so that there can be no question of fusion taking place. The lower row of tentacles of *Pennaria* persists somewhat longer than do those on the hypostome, but both ultimately disappear, and in neither form is there a withdrawal into the stalk until the polyp has almost entirely degenerated.

The results show that in these three hydroids the method of absorption is the same. No trace of liquefaction of protoplasm, or of withdrawal of the polyp as a whole can be found. The absorption takes place by the degenerating cells of the endoderm and ectoderm being turned into the digestive tract of the colony.

Vol. IV. *February, 1903.* *No. 3.*

BIOLOGICAL BULLETIN.

NOTES ON THE STRUCTURE AND DEVELOPMENT OF EMBIA TEXANA.

AXEL LEONARD MELANDER.

Delays in the spring rains of 1902 made Pease Park at Austin, Texas, an unusually good collecting ground for the entomologist during the early summer. Numbers of rare and interesting insects were flying about the *Sapindus* and *Eisenhardtia*, plants which grow profusely there. The now-running Shoal Creek had its share of swarming Ephydridæ. On the open ground in the cedar brakes several ants and a number of Microhymenoptera belonging to genera new to this country were obtained. In the midst of this profusion of insect life we were not greatly surprised on turning over a chance stone to discover the male of *Embia texana* on May 10. This was soon followed by the other forms of the species, so that now we are able to throw some light, at least, on the transformations of these insects.

The question of the sexes of these insects has long been an enigma. Owing to the fact that many of the species were taken in abnormal situations, as on exotic plants imported by nurserymen, but little has been brought to light regarding their habits. Winged, half-winged and wingless specimens of the different species have been taken. Even after dissection the winged ones were affirmed to be females in some species and males in the others. The nymphs were once supposed to be micropterous adults, corresponding with the neoteinic royal forms of the Termites. Of the wingless casts, some had the symmetrical abdomen of the female (larvæ and adult females) and some the asymmetrical genitalia of the male. Inasmuch as a complete series of practically but one of the species has been obtained, and this form has not been altogether correctly interpreted, it is not surprising that this disagreement existed.

The fortunate discovery of all the forms of the American species reveals the fact that the Embiidæ may be polymorphic. During the winter and spring our species exists in the larval state, with occasionally a chance female surviving the summer and living into the autumn in the same nest with her young. In early May the final moultings occur. At that time the larvæ destined to become females increase rapidly in size, and with the final moult their reddish mottled color becomes a dark chestnut brown or even a bronzed black posterior to the prothorax. No traces of wings are evident nor is there a lengthening of the antennal joints. The abdomen of the female retains the symmetrical termination of the larva, is covered apically with longer and denser hairs, the last ventral becomes longitudinally split, and the cerci remain each two-jointed and short. These large females are sluggish in movement, carrying the abdomen with a dorsal hump as does the larva. They still spin their web-nests with glands unaltered during their metamorphosis. They are often social and frequently live two or three in a nest, whereas but a single male develops out of a brood of larvæ. The same fact has been observed in the case of *insularis*. However, in all collections of the Embiidæ the females seem to be much the rarer sex.

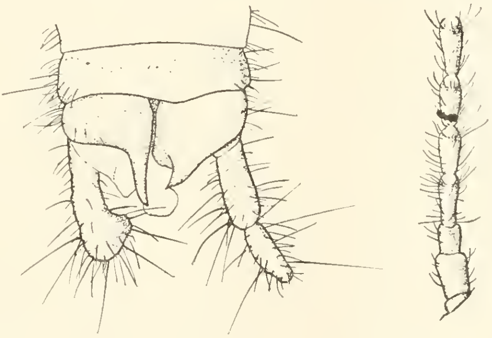


FIG. 1. *Male.* Tip of abdomen, and base of antenna.

With the male the metamorphosis may proceed in one of two ways, *i. e.*, the males of *texana*, and of other species also, are dimorphic. At the penultimate moult wings may be formed, as in the Orthoptera, or not. In the former case a true nymph-stage is attained. The body is but little larger than the full-grown larva, still of a pale reddish color, and marked with the same designs of irregular pigmentation. The antennal joints have the same magnitude and the cerci are two-jointed. Like the larva and adult female the abdomen is carried with the end bent down. The conspicuous change undergone at this moult is the acquirement of wing-pads. These are outgrowths from the anterior and lateral angles of the meso- and metathorax, which are folded over the dorsula, meeting in the median line, and attached by the basal part of their inner margin to the thorax, as in all true Orthoptera, and not as the misleading figure by McLachlan[1] would indicate. This stage has been observed in *insularis*, *Michaeli* and *Uhrichi*. Nymphs of *mauritanica* also have been found by Mr. Nathan Banks on date-palms imported from Algeria to Washington, D. C. The nymph-stage is of short duration, lasting probably not more than a week. Towards the end of that time the outer cuticle begins to separate. The single-jointed left cercus can be seen through the loosened chitin, extending into the second joint and reminding one of the figures of the Forficulid *Dyscritina*.[2]

With the last moult the habitus of the insect changes surprisingly. Full pigmentation and chitinization soon set in and the body becomes jet black with a bronzed tinge. Instead of the cylindrical form of the larva, the abdomen becomes depressed, and is now carried with the tip curved high over the back, altogether different from its former behavior. The front legs become inactive, and except during excitement, are never used for locomotion, being held helplessly in front of the body. The spinning glands of the front metatarsi do not cease to be functional, for the males were observed to spin, though always in a reluctant manner. The antennal joints lengthen, giving to these organs an increase of one half their former extent. The wings now become large, exceeding the tip of the abdomen, and also acquire their full pigmentation and villosity. When not in use they are carried flat over the back. As has been noticed for the other species the wings are attached firmly to the thorax and are in no manner deciduous. Hence the wingless male described below cannot be considered as a dealated form. Herewith is given a more detailed characterization of the male. Aside from the description of the wings it will apply for the wingless form mentioned later on.

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[1] *Jour. Lin. Soc. London*, XIII., Pl. Fig. 2*a*.

[2] See Green and Burr, *Trans. Ent. Soc. London*, 1898, Pl. XVIII.

EMBIA TEXANA Melander.

Male.—Length 6 mm., length of antenna 3 mm., length of wing 4.5 mm. Clear-cut, slender species. Body black, with violaceous or bronzed reflection ; head, prothorax and front femora castaneous brown, middle and hind legs sometimes also brown, but darker ; black-villose, the hairs variable in length and number. Antennæ black, the individual joints pale on their outer fifth, seventeen joints present, and these equalling a little more than one half the body-length, *i. e.*, reaching to the end of the metathorax, basal joint stout, cylindrical, a little longer than wide, second joint of less width, quadrate in profile, the remaining joints elongate-pyriform becoming elliptical terminally, the individual joints about as long as the basal two ; all the joints are provided with stiff radiating black hairs. Palpi of same structure as in the larva, the maxillary palpi black, the labial reddish at the base. The lighter spaces of the head and thorax of the larva are much altered, becoming faint and indefinite in the adult. Propleuræ with two small sharp black tubercles in front of the coxæ. Thorax and abdomen as in the larva, but the chitinization and pigmentation much more advanced ; penultimate segment (ninth including the segment mediaire) narrow, somewhat bullate dorsally towards the right ; ultimate segment asymmetrically bisected dorsally, each portion produced more or less conically and pointed, in the middle of this armature, between the two titillatores a fleshy process may be exserted. Last ventral segment triangular, simple, but the left side is somewhat excised. The dorsal structure is not visible from below. The left cercus large, single-jointed, clavate, obliquely truncated apically, the right appendage two-jointed, with its outer joint shorter than the inner, both appendages hairy. Wings brown, marked with five secondary veins of wine-red pigment, the second and third connected by two to four cross bands of pigment, the third and fourth sometimes also connected. The central space of the cells is clear hyaline.

In this species the venation of the wings is much reduced, only the base of the subcosta, the radius, the cubitus, and the indefinite base of the anal vein exist as thickened cuticle ; the original venation is represented by bands of wine-red pigment arranged like lines of granulations and by series of short hairs. These lines extend just posteriorly to the cuticular thickenings and represent the full venation of the nymph, except that the costa is wanting in the adult. Owing to this double venation, which, judging from the figures, occurs also in a number of the other species, there has been much confusion in homologizing the veins with those of other insects. On applying the ontogenetic method of Messrs. Comstock and Needham, which fortunately the possession of nymphs of two species enables us to do, it will be seen that the interpretation given by these gentlemen of one of Wood-Mason's figures is nearly correct. It might be suggested, however, that the cross-veins are not to be regarded as branches of the radius, but as additional veins, for their position is very unstable, and moreover, in the nymph they are indicated merely by a sinuosity on the radius, very much as in *Psocus*,[1] from which type they do not materially disagree. *Texana* has the simplest type of true neuration of any of the species of this family, the veins being reduced to those thickenings mentioned before. Figures of its nymph-wing, which is also identical with that of *mauritanica*, and of the adult wing of *Uhrichi* (copied from Saussure)[2] are given for comparison. In the latter species the neuration reaches its highest degree of complexity.

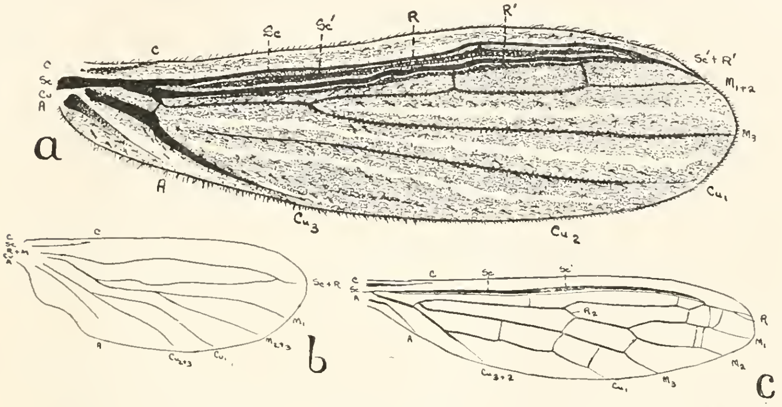

FIG. 2. *a*, wing of adult; *b*, wing of nymph; *c*, wing of *Embia Uhrichi*.

If before the final moult wing-formation does not take place, the adult stage is attained in a wingless condition. These wingless males are more frequently met with in the nests than the winged form, though never more than one was to be found in a single nest, which often would contain several females. In color, size and structure both forms of the males are exactly similar. Moreover, they have the same movements, with forelegs elevated, though the wingless male generally carries its abdomen curved further over the back. Their only difference is in the matter of wings.

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[1] *Vide, American Naturalist*, XXXII., p. 241, Figs. 11 and 12.

[2] *Mitt. d. Schweiz. entom. Gesell.*, 1896, Pl. Fig. 1.

In the *Entomologists' Monthly Magazine* (1897, p. 56), Mr. R. C. L. Perkins notices a peculiar condition of the thorax of *Embia insularis*. From the hinder angles of the meso- and metathorax project small lobes which he concludes are the beginnings of the wing-pads at the stage immediately preceding the nymph-form. A specimen of a full-grown wingless male of *texana* presents the same peculiarity. This cannot be explained as a case of atavism — an attempt at the wing-formation which this form has lost — for the true wings arise from the anterior angles of these segments and hence are not homologous with these free lobes. Moreover, we have sectioned the fully-grown larva and find the wings as large invaginated pockets completely beneath the hypodermis.

The males of *Solieri* seem undoubtedly all to be wingless, although the evidence rests largely on the absence of winged forms. Professor Grassi and Dr. Sandias obtained numbers of mature wingless males but none with wings. However their observations were interrupted during July to September.

The structure of the anal cerci of the Embiidæ varies with the individual. The males of three species (*tartara*, *texana* and *Wheeleri*) have the left cercus always single-jointed and the right one two-jointed. As far as can be judged from the descriptions all the other species (except possibly *mauritanica*) have both appendages two-jointed in the male as well as in the female. It is among this latter class that the structure of the cercal joints varies.

Herewith is appended a table of all the described species of this family, indicating the extent of polymorphism as far as has been recorded. The localities of distribution are added to accompany the map given later on.

	larva.	♂ nymph.	♂ wingless.	♂ winged.	♀ wingless.	
1. *æthiopicorum* Karsh.				×		N. Camerun, W. Africa.
2. *agilis*[1] Sundeval.	×					Bahia, Brazil.
3. *antiqua* Pictet & Hagen.			×			Prussian amber.
4. *Batesii* McLachlan.				×		Brazil.
5. *bramina* Saussure.				×		Bombay, India.
6. *braziliensis* Gray.				×		Brazil.
7. *cubana* Hagen.	?			×		Cuba.
8. *hova* Saussure.				×	×	Madagascar.
9. *Hubbardi* Hagen.				×		Enterprise, Florida.
10. *humbertiana* Saussure.				×		Ceylon.
11. *insularis* McLachlan.	×	×	×	×	×	Honolula : Antigua Isl.
12. *mauritanica* Lucas.	×	×		×		Algeria.
13. *Michæli* McLachlan.		×	×	×	×	Ambulla, Calcutta, India.
14. *Muelleri* Hagen.					×	Sta. Cattarina, Brazil.
15. *nigra* Hagen.				×	×	Upper Egypt, Cairo Egypt : Kellemisch, Asia Minor.
16. *nobilis* Gerstecker.						Itaituba, Amazon, Brazil.
17. *persica* McLachlan.				×		Shahrud, N. Persia.
18. *ruficapilla* Burmeister.				×		Venezuela : Brazil.
19. *ruficollis* Saussure.				×		Central America.
20. *Salvini* McLachlan.				×		Tehuantepec, Mexico : Chinautta, Central America.
21. *Saundersii* Westwood.			×	×		Bengal, Jubbulpore, Calcutta and Bombay, India : Borneo : Mauritius : Madagascar : Ascension Isl.
22. *Savignyi* Westwood.				×		Egypt : Greece : S. Russia?
23. *Solieri* Rambur.	×		×		×	Spain : France : Italy.
24. *tartara* Saussure.				×		Turkestan.
25. *texana* Melander.	×	×	×	×	×	Austin, Texas.
26. *trinitatis* Saussure.	×			×	×	Trinidad.
27. *Uhrichi* Saussure.		×		×	×	Trinidad.
28. *Westwoodi* Hagen.				×		Zanzibar? copal.
29. *Wheeleri* Melander.			×			Cuernavaca, Mexico.

That the wingless males are functional is to be seen from the following interesting observation. On June 1 a male that had been kept in captivity for some time was admitted to the dish containing some isolated virgin females. At once every action denoted an intense excitement. The quivering of the antennæ, the tremors of the body, the hurried runs hither and thither, and the gnawing at the web, were all strangely in contrast with the passive demeanor in the former nest. His entire behavior indicated that his senses perceived the proximity of the other sex. At last a run brought him beside one of the females. A sudden

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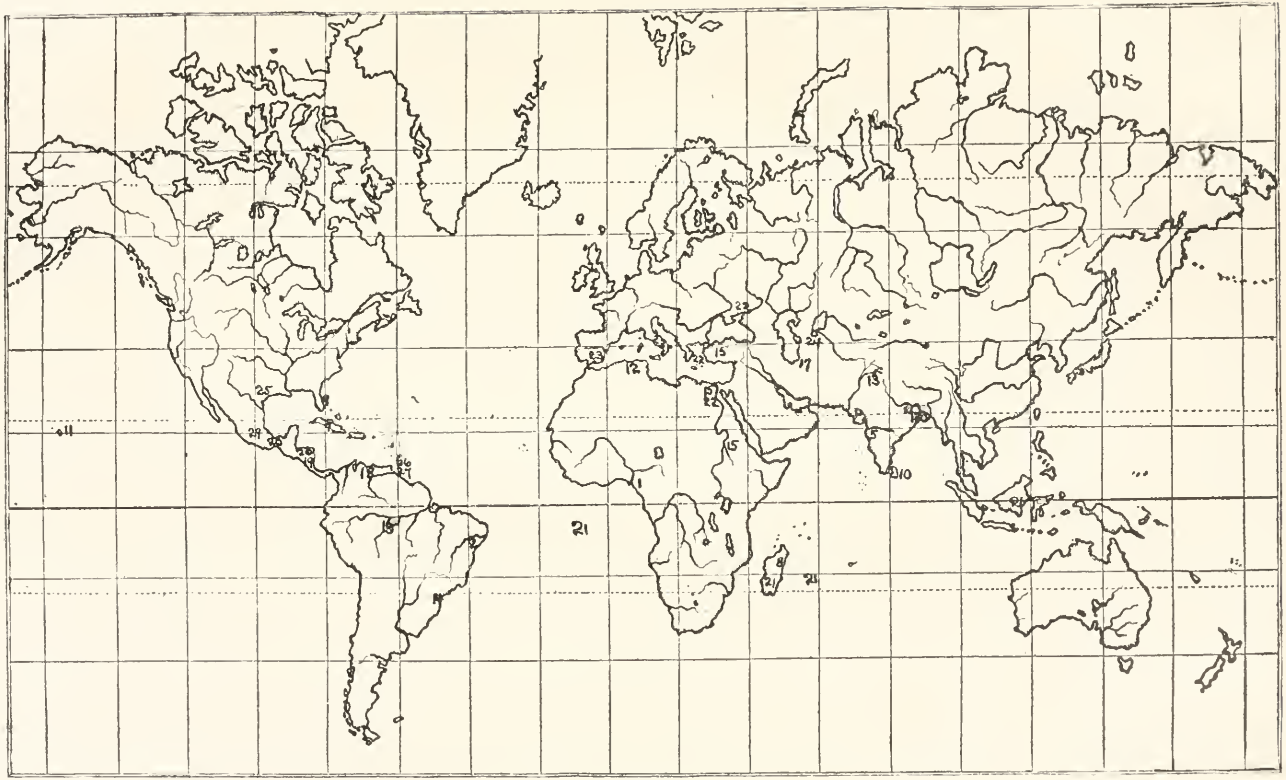
[1] This species, described as the larva of a new genus of Forficulidæ, *Condylopalama*, will probably never be recognized from the brief description.

calm overcame him, and with his mouth and the fore feet that normally are so inactive, he caressed the head and thorax of his bride. This lulled her to tranquility, and with a sudden dart the male turned around and clasped the eighth ventral in his bifid pygidium. Instantly both were struck with a rigor. They allowed themselves to be turned over without showing a sign of movement, and were it not for the rapid but faint pulsation of the thinner chitin of the segmental interstices of the male they would have seemed as if dead. For four and one half minutes they remained thus, the body of the male twisted over the back of the female, and turned towards her right side. The copulation then being effected they separated, and both ran about with the quiet movements of their ordinary gait. Now we understand why the male so frequently carries the tip of the abdomen uplifted for this position has a functional significance during coition. During this time the heart-beat of the male attains three hundred per minute. This pulsation is remarkable when we remember that the highest number recorded is one hundred and forty-two per minute for *Anthophora*, and that, too, at a time of excitement.

Distribution.

All of the specimens of *texana* were taken in the same situations as were the larvæ described in the June number of this periodical, under flat limestone surface-rocks that form so prominent a feature of this region of the Texas country. Net-sweepings through the grass in the vicinity failed to reveal any specimens. This is to be expected, however, for our species is practically nocturnal. In the case of *mauritanica*, specimens have been taken in grass-sweepings during the daytime, while *nigra* has been found in the same way in the evening. The situations frequented by the different species present quite a range of variation. Some have been found among the roots of orchids where they are said to cause considerable injury (*insularis*, *Michaeli*, etc.). Many construct their web-nests on the fibrous bark of palms and cycads (*Saundersii*, *mauritanica*, *Uhrichi*, etc.). The former species, too have been seen running about on the sand. The habits may vary with the altitude, even. *Insularis* becomes arboreal in high situations but is found under stones in the low-


11
25
21
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21

Fig. 3.

lands. During the night the males of this species fly freely to lights. A similar habit has been noticed with the Indian species.

All of the species, however, require a very definite degree of moisture and hence are restricted in their distribution along the seacoast. A glance at the accompanying map will make this evident. Here are plotted the known *patriæ* of the various species, numbering the localities to correspond with the list of species given before. Where the identification is doubtful a cross expresses the provenience. A comparison of the available and recognizable descriptions of previous authors brings to light several facts. The old group *Olyntha* (represented by species 25, 29, 20, 27, 6, 18, 4, 14, and probably by 19 and 26) though untenable as a genus, yet is comprised wholly of American forms. The only other American species, *Hubbardi* and *cubana* are related among themselves and very closely with the Sandwich Island species, *insularis*, which is said to occur in the West Indian island Antigua also. Moreover, *insularis* is a near relative to *Saundersii* (21) which has an extended distribution over Borneo, India, Mauritius, Madagascar and Ascension Island. With this species are grouped also the Indian species, *Michaeli* (13) and *bramina* (5), and possibly also *humbertiana* (10, from Ceylon) and *hova* (8, Madagascar), themselves closely allied and forming small groups. *Mauritanica* (12) and *Savignyi* (22) are stated to be close relatives, and both live along the southern Mediterranean coast. *Nigra* (15) of Egypt is represented by a variety (?) in Asia Minor. Thus we find a number of groups, natural both in structure and in distribution. The New World forms, excepting those of the West Indian fauna, which probably are derived from an oriental source, stand out alone. *Embia Solieri* (23) of southern Europe has little affinity with the other species, its nearest relative being *antiqua* (3) from the Prussian amber, of Tertiary times, a form also with wingless male, and not clearly distinguishable from the living species. India seems to be the point of expression of another group of species (species 13, 5, 21, 10); of these *Saundersii* (21), or forms indistinguishable from it, occurs with a wide island-distribution. It seems to be with these species that *cubana* and *insularis* are to be grouped. Australia, eastern Asia and western South America are, as far as known, destitute of species. The Embiidæ are therefore seen to be tropicopolitan and also seacoast species, extending only a few hundred miles into the interior along the principal water-courses. In the warmer countries they appear at higher altitudes, in order to preserve conditions of proper temperature and humidity. As the species show a preference for palms and orchids, plants which have a commercial importance, it is not surprising that their distribution is artifically complicated. At least four species have thus been taken in ship-yards and green-houses thousands of miles from their native homes. Moreover, a natural maritime distribution is quite possible on tree-trunks swept down some river during flood-time and then carried about by ocean currents. *Texana* has actually been found under the bark of a fallen tree lying on a flood-plain. The next heavy rain would have carried that tree-trunk down the Colorado River and ultimately to the Gulf of Mexico. These facts should be remembered when we consider the wide distribution of *Saundersii*. It might be mentioned that like so many other introduced animals this species has become exceedingly abundant in some places, even to a source of annoyance in the island of Ascension. *Insularis*, too, is said to be common on the sea-shore, every stone serving to shelter two or three. This fact might indicate an introduction during times not distant, with conditions favorable for a rapid increase in numbers, and only recently an extension into the interior.

That the Embiidæ form an ancient group is undisputed. Their uniformity of structure, so marked that characters of specific importance are few, shows that they have long passed the zenith of their evolution. The variability of the antennæ and of the cerci, and even of the neuration of the wings might suggest a decadence of these organs. The fact that the males are dimorphic and have only partially the need for wings, retaining them possibly only to prevent too close interbreeding, while the females are all wingless, also would lead us to that conclusion. But the Embiidæ have existed a long time in this dimorphic condition of the male, for the wingless form is known in amber from the Tertiary. This family of frail insects, one of the first of the twigs of the great Orthopteran branch, seems certainly in process of extinction, but like other groups which were conservative in specialization, they too are destined to outlive many of their later but more plastic companions.

International Anatomy.

The forepart of the alimentary canal consists of a small buccal cavity, a narrow pharynx, dilating into the large œsophageal tract, which extends into the metathorax. The central part of the œsophagus is narrowed, as in *Solieri*. At the hinder end of this portion there is formed by a sudden constriction a narrow tract which terminates that part of the alimentary canal of stomodæal origin. Thus far the tract is lined with a chitinous cuticle presenting differently formed teeth along its course, and is enclosed within a double layer of muscles — the inner layer longitudinal, the outer circular — which become stronger posteriorly. The teeth of the buccal cavity are closely placed, long, directed backward, and attached in sockets. In the pharyngeal constriction they become minute and flattened, more closely aggregated, still directed backward, but appearing like small ctenoid-scale-like projections of the cuticle. Below the frontal ganglion of the sympathetic system the scales are wanting but recommence further on. The pharynx is thrown into eight strong longitudinal folds, of which two are lateral, two ventral, and four dorsal. This gives to a trans-section of the pharynx a hexagonal, not octagonal, appearance. Within the proventriculus the teeth are reduced to mere granulations of the cuticle, arranged in narrow scattered areas.

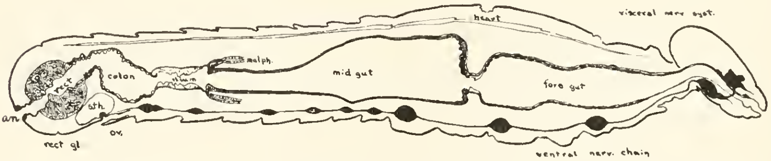

Fig. 4. Diagrammatic arrangement of the body-organs in a medial sagittal section. *an*, anus; *malph*, malpighian tubule; *ov*, oviduct; *rect*, rectum; *rect. gl*, rectal gland; *sth*, spermatheca.

The mid-intestine commences as a sudden enlargement more or less telescoping with its pedicel, and which gradually narrows to the point of attachment of the Malpighian tubules in the sixth segment of the abdomen. Its walls consists of columnar epithelium — short uniform cylindrical cells in the larva and elongate, more or less pear-shaped, irregular, secreting cells in the adult. The muscular layer surrounding the mid-gut is much reduced, and the chitinous lining of the fore-gut is wanting in this portion. At the base of the Malpighian tubules the wall of the mid-intestine is thickened, forming the gastro-ileal valve. Here, in a conical chamber formed just beyond the valve, commences the cuticle from the proctodæal invagination, but unlike the œsophageal chitin, it is thin, replicated and smooth. The ilium is normally much contorted, but when filled with food can be straightened. Its numerous circular muscles then force it tense and crowd the mid-gut into the thorax. The globular rectum fills out the last two segments of the abdomen. When not evacuated it is filled with rasped wood and other fragments of vegetable origin. Its walls are strongly bulged by the six large cushion-like rectal glands which consist of long convoluted secreting (?) cells each with a large central nucleus. The Malpighian tubules vary in number with the age of the individual, becoming about twenty-four in the adult. In the younger stages they are rather shorter and thicker with their nuclei more aggregated, *i. e.*, their cells are smaller. Their cells frequently present a honey-combed structure, probably due to the solution of their contents in the reagents for clearing.

The heart of *Embia* can be distinctly seen through the overlying translucent chitin, extending the length of the body. Even when pigmentation is greatest the heart is still evidently visible from above. The blood corpuscles are large and elliptical.

The supraœsophageal ganglion (brain) of the nervous system is relatively small ; the optic lobes send off a stouter nerve to the eyes than is apparently the case in Professor Grassi's species. This may be in part due to a difference in the power of sight in the two species. The deutocerebrum is flattened, the antennal nerves very slender, an innervation that might be expected for organs of so poor sensory development. Owing to the absence of ocelli there are naturally no ocellar nerves. The subœsophageal ganglion is comparatively large, sending an anterior nerve in front of the œsophageal commissures. The remainder of the nervous system is nearly similar to that of *Solieri*, consisting of three large thoracic ganglia and seven smaller abdominal ganglia connected by the double ventral cord. The fifth segment is, as has been noticed in *Solieri* and *Uhrichi*, destitute of a ganglion, the ventral cord between the fourth and fifth ganglia being longer than between the others. But in our species the last ganglion (seventh abdominal) is crowded forward, though still remaining larger than the other ganglia of the abdomen. Its position is above the seventh sternite and contiguous with the ganglion in front. In the two species before compared the last ganglion is connected with the sixth by a commissure and is placed in the eighth segment. Each of the ganglia send side branches into the viscera, while the last terminates in a pair of nerves interwoven with the efferent sexual ducts.

The visceral system is somewhat different from that of *Blatta*.

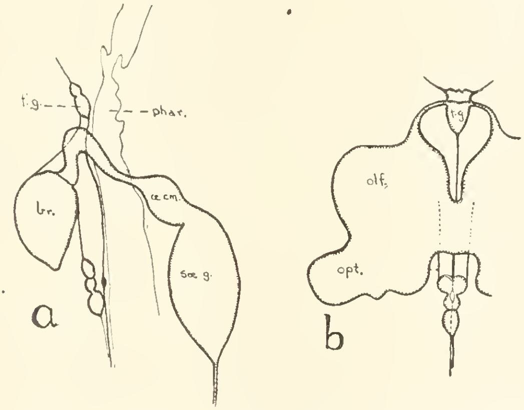

FIG. 5. *a*, lateral; *b*, dorsal view of the cephalic nervous system; *br.*, brain; *f. g.*, frontal ganglion; *œ. cm.*, œsophageal commissure; *olf.*, olfactory lobe; *opt.*, optic lobe; *phar.*, pharynx; *sœ. g.*, subœsophageal ganglion.

It consists of an unpaired triangular frontal ganglion of rather large size, connected laterally by stout commissures with the anterior lobes of the brain. The lower portion of this ganglion ends laterally in nerves extending obliquely forward along the œsophagus. The frontal ganglion ends posteriorly in a long slender nerve lying immediately above the coat of circular muscles of the alimentary tract, and enlarged as two ganglia over the œsophageal portion, one within the prothorax, the other in the mesothorax, though still lying close over the œsophagus. At intervals this nerve sends off a series of fine nerves anastomosing in minute knots and which terminate within the circular muscles of the tract. The remaining ganglia are unpaired also, lying in the median line above the alimentary tract and within the hind fork of the brain. These are three in number, more or less fused, and the anterior one bilobate. They send but one slender nerve backward, above the one from the frontal ganglion, and are connected with the brain by two commissures arising from the sides of the anterior bilobed ganglion. As the peripheral nerves of this system intimately innervate the muscles of the alimentary tract, conforming with all its irregularities, it would seem that peristalsis is controlled from this source. Professor Blandford did not find the posterior ganglions of the visceral system of *Uhrichi*. This was probably due to their small size in the specimens studied rather than to their complete absence. All the ganglia vary in size almost more than do the other organs of the body. In some cases the posterior ganglia of the visceral system are much reduced and flattened.

The female generative organs agree with the descriptions of those of *Solieri* and *Uhrichi*, consisting of a paired oviduct, each branch of which emits five lateral tubules. In the larval state the tubules are consolidated into a compact elliptical mass, situated dorsally one to each of the five basal segments of the abdomen. Each mass comprises about seven transverse chambers and is connected with the slender oviduct by a short and equally slender strand. In the adult the tubules lengthen by a forward growth, the chambers separate and on becoming elliptical give a moniliform appearance to the tubule. The end-cells remain undifferentiated. In a multiparous female the oviduct becomes greatly distended and its walls much wrinkled. This distension is evident also at the base of the recently emptied follicles. The spermatheca is large, occupying the free portion of the eighth segment. It opens below by a somewhat tortuous duct into the short vagina. The end of the ventral nerve chain passes between the vagina and the spermatheca.

EMBRYOLOGY.

During the few weeks the Embias were kept in captivity the females deposited a small number of eggs. These were laid during the night-time, generally one at a time, and were often insecurely fastened to the surface of the jar by strands of silk. The eggs, a dozen or so in number, were carefully preserved in hope that the history of the embryos would give some clue as to the systematic position of the family.

The eggs are of a creamy-white color and of the retort-shape noticed in *Solieri* and *Uhrichi*, and possess a characteristic narrow circular band of thickened chitin extending obliquely from the apex of the narrower end to one third the distance along one side. This structure is of great service in orienting the egg during the later stages. In the ovaries of the female the eggs lie with the narrowed end anteriorly and the oblique surface directed upward and towards the median line of the body. Thus the primitive ventral surface does not correspond with the definitive, but the anterior end of the ovarian egg remains the anterior end for the hatching embryo. The oblique circle outlined by the ridge is doubtless detached at the time of hatching. The chorion is thin but firm, and presents a smooth finely granular surface. Within the circle, however, the surface is lightly roughened by trabeculæ and minute pillars, its alveolar structure corresponding with the enlarged cells of the follicular epithelium. At the posterior median point of the ridge is a small elliptical opening, the micropyle, communicating with the irregular lumen of the ridge itself. This opening is in connection with a smaller one passing into the interior and provided with a grooved guideway. As in *Blatta*, no gyration of the egg is necessary, during the passage down the oviduct; the micropylar opening comes directly in contact with the mouth of the spermatheca, while the small size of the opening limits the number of spermatozoa that find ingress. The chorion is sometimes thin, due to imperfect secretion, and at times is covered by the shed follicular epithelium. In this detritus and among the strands of silk particles of soil adhere. This might indicate that in the natural nests the eggs are concealed for protective purposes by an added covering. The vitelline membrane is comparatively tough and separates from the chorion in eggs kept in an alcoholic preservative.

Unfortunately the youngest embryos were well advanced at the time of preservation, being in an elongate fully-segmented stage. As the embryo now extends over the whole of the definitive dorsal surface with its head directed posteriorly, it probably attained this position by rotation, keeping at the surface of the egg as in the Termites and not plunging through the yolk as in the Orthoptera with elongate eggs. At this stage segmentation is nearly complete, only the posterior end being confused. The antennæ are short, borne by the rather large deutocerebrum which shows a flexure around the posterior pole. The mouth parts are large, single-jointed appendages, the labrum distinctly bipartite. The head segments are nearly as strongly marked as the thoracic. Of the limbs the first pair are slightly more advanced than the others. Whether this is due to the antero-posterior direction of growth or to an acceleration of these important appendages would be hard to decide. The pleuropodia are large, the remaining abdominal appendages are uniformly smaller and disappear on the fifth segment. Beyond this segment the abdomen curves into the yolk, while at the eighth segment there is a sudden outward flexure. The "tail-piece" thus formed is quite like the similar growth of *Termes*, etc. Sections of the

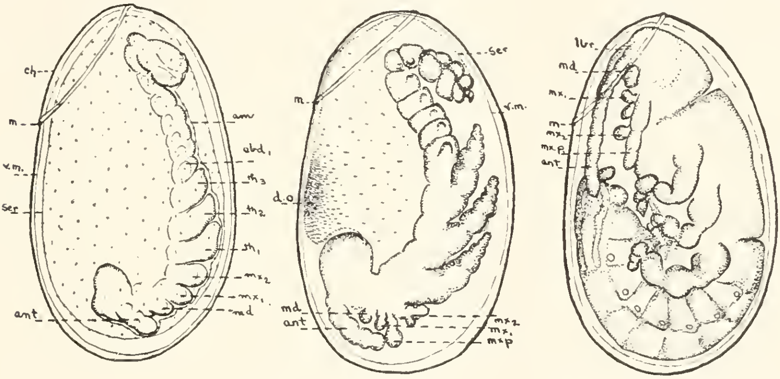

FIG. 6. Three stages of the developing embryo. *am*, amnion; *ant*, antenna; *ch*, chorion; *do*, dorsal organ; *lbr*, labrum; *m*, micropile; *md*, mandible; $mx_{1,2}$, maxillæ, *mxp*, palpus; *ser*, serosa, $th_{1,2,3}$, legs; *v. m*, vitelline membrane.

egg reveal only the usual envelopes over the embryo, *i. e.*, the chorion, the tough vitelline membrane, the serosa with large nuclei, the amnion with smaller more rounded nuclei, and the blastodermhaut. The yolk of these eggs, fixed by hot water, shows a definite segmentation, a dozen or so of granules being aggregated about each vitellophag.

During the next few days the embryo changes rapidly in appearance, due to the process of shortening, *i. e.*, broadening. The appendages lengthen, the mouth parts take on their final structure and position, the maxillæ becoming tripartite, much like *Termes*, due to the development of their palpi. The head, however, still continues its accelerated development, but lies free from the yolk. The abdominal legs still persist, though even yet, except for the terminal styles, none are formed on the "tail-piece," which is more flexed than before and in which the proctodæum is seen in process of formation. The serosa and amnion have parted over the embryo and are drawn back as the "dorsal organ."

The last stage of which we have material shows a marked advance in development, although the embryo is but three weeks old. Revolution has now occurred. The envelopes, except the vitelline membrane, as well as the dorsal organ, have disappeared. The embryo has grown over the yolk, but the dorsal abdominal cuticle is still thin and shows its derivation from the serosa by scattered nuclei over its surface. The proctodæum and stomodæum are well formed but still are separated from the yolk-mass. The head-lobes have dwindled to a microcephalic size, the antennæ have lengthened to the base of the fore legs, but even now the maxillary palpi are nearly as long; yet the antennal segmentation is more marked than in the corresponding stage of *Xiphidium*; the labrum is large and separate; the eyes are not yet formed. The tarsi are now suddenly flexed outward, with the front metatarsi distinctly large. The abdomen is stout and much bent on itself. The appendages, except the cerci, have disappeared, and in their stead conspicuous spiracles are developed.

From the rapidity with which these stages are passed through it would seem as though the embryonic life of *Embia* is of short duration. A development at this rate would cause hatching to occur in late June, at the very height of the dry season. But as these frail insects cannot undergo heat and drought, as has already been shown, it seems not improbable that soon after the last stage described above there is an arrest in development which enables the embryo to æstivate within the protecting chorion until the rains of later summer. Under the conditions of Texas weather it does not seem likely that there are two broods a year. Moreover, from autumn till spring there is a gradual increase in the size of the larvæ observed.

The few stages obtained show nothing but what might be expected in the development of this insect, and simply indicate that the position of the Embiidæ in the superorder Orthoptera is doubtless correct. The latter part of the embryonic history, at least, shows a closer parallelism with the Termitidæ than Grassi's conclusions drawn from the adult structure would lead us to expect. At any rate, the comparatively large size of the head of the young embryo, the persistence of the abdominal legs and the structure of the appendages and of the "tail-piece" might have some phylogenetic bearing. The late formation of the eyes, the enlarged front metatarsi, the relative sizes of the antennæ and maxillary palpi are marks of specialization, indicating arrested or precocious development, and are not to be sought for beyond the ontogeny of this form.

Summary.

The females of the Embiidæ are comparatively large, wingless and symmetrically formed, the males have the abdomen more or less distorted (except species 17, 18 and 22). The males of probably all the species are dimorphic, being wingless (not dealated) or winged. The wings arise from invaginations at a pre-nymphal instar. A nympha-stage with larva-like body is undergone. Venation varies with the species, and generally is double, represented by true veins and pigment lines. The thickened veins of *texana* are few. Copulation occurs within the nests. The carriage of the male abdomen is attributable to its function. The facets of the Embiid eye are quite like those of a newly-hatched grasshopper.

The entire family is tropicopolitan and prefers the humidity of the coast-region. Its extended island-distribution appears to be in part artificially effected. Brazil, the Mediterranean region and India seem to be the points of differentiation of the groups *Olyntha*, *Embia* and *Oligotoma*, respectively.

The internal anatomy presents a generalized type. But little change is undergone during metamorphosis. The oviducts of an old female may be as large as the alimentary tract. The females have all available space occupied by the fat body and later by the developing eggs. The brain is much less developed than the ventral nerve chain. The visceral system comprises four prominent unpaired cephalic ganglia.

Embryonic growth is completed in about a month. The eggs have a unique micropylar apparatus and are not oriented in the female in their definitive position. The history of the embryo conforms with the Orthoptera, of which group the family is considered as a conservative and ancient branch.

HULL ZOÖLOGICAL LABORATORY,
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A NEW SPECIES OF NEMERTEAN (*Cerebratulus melanops*) FROM THE GULF OF ST. LAWRENCE.

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Yale University.

Among a small number of Nemerteans collected by Dr. J. Schmitt at Anticosti was a single perfect individual of a species of *Cerebratulus* which is apparently undescribed. This specimen is of about the size and proportions of medium-sized individuals of *C. marginatus* which probably occurs in the same region, but from which the present species can easily be distinguished by the color and by the possession of several distinct ocelli on each side of the snout. *C. grœnlandicus* Punnett has recently been described from Greenland[1] and may also occur in the same region as the present species, but the absence of ocelli in the former is a sufficient specific distinction. In only a few other species of the genus are ocelli present in the adult worms, although it is possible that they are generally present in very young individuals. Besides the possession of ocelli the species is remarkable because of the presence of a large number of efferent nephridial ducts, although a number of other species, including *C. grœnlandicus*, possess this same peculiarity.

The single specimen on which this diagnosis is based had been preserved in formalin, and may be described as follows: Body elongated, rather slender and ribbon-like, of the general size and proportions of *C. marginatus*. After preservation, rounded in esophageal region, moderately flattened posteriorly, tapering gradually to posterior extremity. Head of moderate proportions as in *C. marginatus;* mouth of large size, elongated, situated as far back as posterior ends of cephalic furrows; proboscis pore small, subterminal; cephalic furrows rather short, separated anteriorly from each other and from proboscis pore.

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[1] *Proc. Zool. Soc. London*, p. 99, 1901.

The single preserved specimen measured about 25 cm. in length and 4 to 5 mm. in width.

There are several *ocelli* of moderate size situated in a single row on each lateral margin of the snout. In the single specimen at hand there are three conspicuous ocelli on each side near the tip of the snout (Fig. 1).

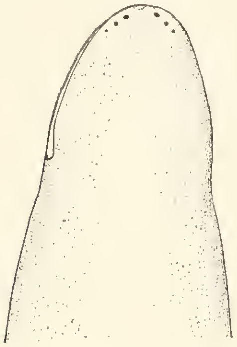

FIG. 1. Dorsal view of head, showing position of ocelli and cephalic furrows.

The *color* of the body is olive green on the dorsal surface, with a darker oval area situated on the dorsal side a little behind the tip of the snout; the ventral surface and lateral margins of the body are much paler or colorless.

In internal anatomy the following peculiarities of structure may be noted:

The *epithelium* is composed of the usual slender, ciliated columnar and glandular cells — the latter containing a secretion which stains deeply and is often found extruded in oval masses among the cilia. The two delicate layers of integumental muscles are arranged as in related species. The *cephalic glands* are limited to the anterior portion of the snout. The *cutis glands* form a compact but comparatively thin layer immediately beneath the underlying fibrous layer, as in many related species, and are therefore distinctly separated from the internal longitudinal muscles by the fibrous layer of the *cutis*. The average thickness of the layer of cutis glands is only about one third to one fifth as great as that of the outer longitudinal muscular layer. As in other species, some of the glands show a peculiar affinity for nuclear stains while others take on the plasma stains only, as has been described by Miss Thompson[1] for *Zygeupolia*.

The *proboscis sheath* becomes very small towards the posterior end of the body (Fig. 3) and terminates some little distance anterior to the anus. The *proboscis* is attached anteriorly to the tissues of the head immediately in front of the brain, but whether the posterior end was attached to the wall of the sheath was not determined. From the conditions found in *Cerebratulus lacteus*,

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[1] *Proc. Acad. Nat. Sci. Philadelphia*, p. 664, 1901.

Zygeupolia litoralis, and certain other Heteronemerteans, it is not unlikely that the retractor muscle is absent. Only two layers of muscles are present in the proboscis, a longitudinal layer on the outside and a circular layer within — the inner longitudinal layer found in many species of the genus being entirely wanting. Inside the muscles occur the usual nervous plexus and the connective tissue layer situated beneath the inner glandular epithelium.

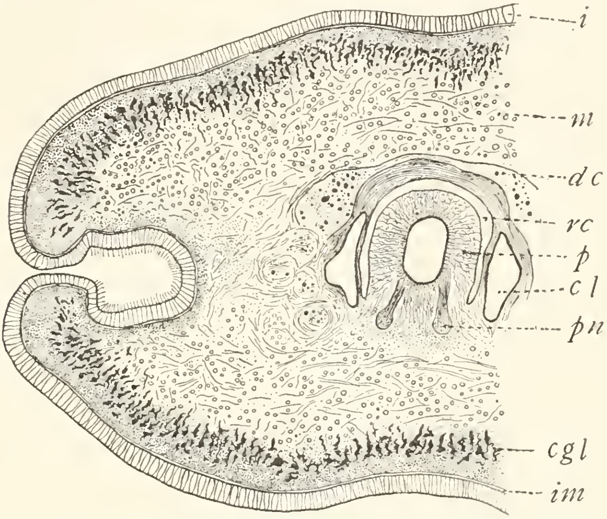

FIG. 2. Portion of transverse section through head in region of dorsal brain commissure (*dc*) and attachment of proboscis (*p*) to tissues of head. The two highly developed proboscis nerves (*pn*) are just on the point of entering proboscis. The disposition of the cutis glands (*cgl*) and their absence beneath the cephalic furrow, the relations of the cephalic blood lacunæ (*cl*) and the cephalic musculature are shown; *i*, integument; *im*, integumental muscles; *rc*, rhynchodæum; *m*, cephalic muscles.

Dorsal and ventral muscular crosses are well developed in this species.

All three of the *muscular layers* of the body walls are well developed. In the posterior portion of the body alone is the internal longitudinal muscular layer very much reduced.

In regard to the alimentary canal it should be remarked that the *esophagus* may be differentiated histologically into an anterior and a posterior chamber, as has been described so fully by Miss Thompson[1] for *Zygeupolia*. The anterior chamber, or esophagus proper, is distinguished by a great abundance of ciliated cells with very numerous short cilia and with their nuclei placed near the superficial border of the cells; while the posterior chamber, or *stomach*, is characterized by fewer ciliated cells, and these have less numerous but longer cilia, the nuclei are pressed nearer the basal portion of the cells, and the gland cells contain a much more granular secretion and vacuolated cytoplasm than those in the anterior chamber. The *rectum* is longer than in many related species, and the *anus* opens on the dorsal surface at the posterior end of the body.

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[1] *L. c.*, p. 706.

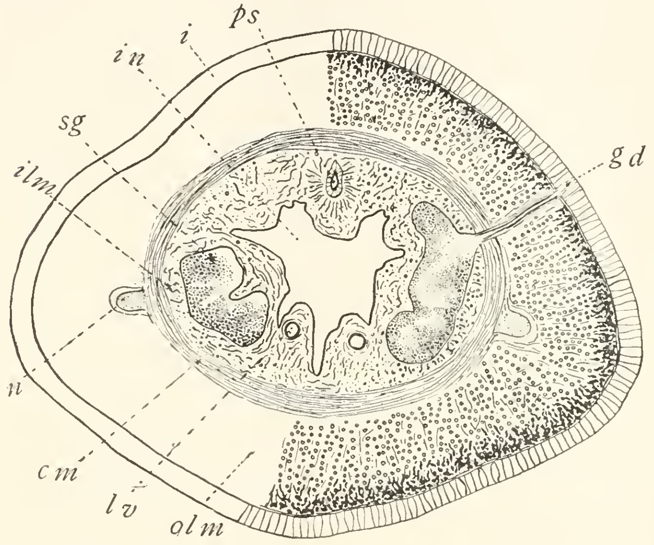

FIG. 3. Transverse section through posterior end of body, showing the very minute proboscis sheath (*ps*), the lateral vessels (*lv*), the sexual glands (*sg*), and the genital duct (*gd*). The internal muscular layer (*ilm*) is here very much reduced while the outer longitudinal muscles (*olm*) remain comparatively massive; *n*, lateral nerve; *in*, intestine; *cm*, circular muscles.

The *blood vascular system* presents the usual arrangement of cephalic and esophageal lacunæ. There is a single broad cephalic lacuna (Fig. 4) situated above and beside the rhynchodæum, and extending from near the tip of the snout almost to the brain region where it is divided into two lateral lacunæ by the enlargement of the rhynchodæum (Fig. 4). These two lacunæ unite ventrally in the brain region to be again separated after a short space. At the posterior end of the brain region they are again united by a broad median lacuna which gives rise to the proboscis sheath vessel posteriorly (Fig. 4). Behind the mouth the lateral lacunæ send off a great many anastomosing blood spaces beside and beneath the esophagus. On the walls of these blood spaces delicate branches of the *nephridial tubules* ramify in all directions. From the pair of nephridial canals which lie on the lateral walls of the lateral blood spaces numerous efferent ducts pass through the muscular layers to the dorso-lateral aspects of the body as usual. These ducts are probably variable in number for in the single specimen sectioned there were thirteen on one side and only seven on the other. In addition to the seven ducts of this side were two incomplete or rudimentary ducts (Fig. 4, *ed*) which did not connect with the nephridial canals at all and are apparently the remains of formerly functional ducts which, for some reason or other, have become degenerated and no longer functional. Similar rudimentary efferent ducts have been noticed in several other forms.

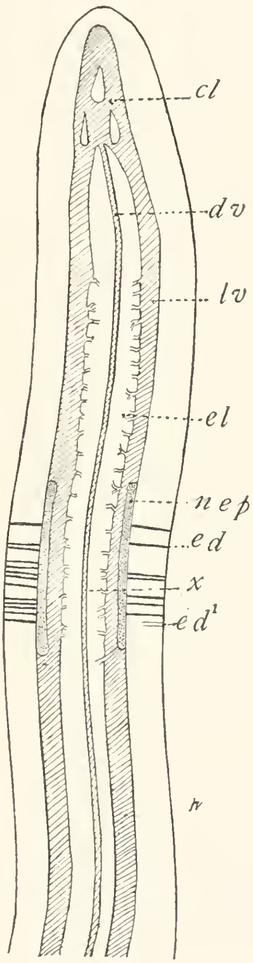

FIG. 4. Diagram of blood vascular system and nephridia. The two cephalic lacunæ (*cl*) unite anteriorly by a rather broad anastomosis. In the brain region are two other anastomoses of the cephalic lacunæ, from the posterior of which the dorsal vessel (*dv*) and the lateral vessels (*lv*) originate. The lateral vessels give rise to profusely branched anastomosing lacunæ (*el*) beside and beneath the esophagus. Dorsal vessel leaves the wall of proboscis sheath in the midst of nephridial region (*x*). The nephridia (*nep*) lie on the lateral walls of lateral vessels about hal way between mouth and intestinal region, and send numerous branches among the esophagal lacunæ. They open to exterior through numerous efferent ducts (*ed*) on each side. In this specimen there were thirteen on one side and seven on the other. There are, however, two rudimentary ducts (*ed′*) on the side with the smaller number.

The *brain and the esophageal*, *dorso-median* and *lateral nerves* are as in related species. The *proboscis nerves* of which there is a single pair arising from the ventral ganglia, near the origin of the ventral commissure, are remarkably large and conspicuous. As seen in Fig. 2, they enter directly into the ventral wall of the proboscis at its attachment to the tissues of the head (immediately in the region of the dorsal brain commissure) and then pass into the midst of the longitudinal muscles of the proboscis where they divide into a number of smaller nerves. A short distance behind the brain these spread out into a plexus beneath the epithelium, as in other species in which the inner muscular layer of the proboscis is wanting. The three slight pits on the tip of the snout, representing the *frontal sense organs*, are comparatively well developed. *Cerebral sense organs* are also highly developed, and exhibit the usual structure and relations with the dorsal ganglia and the cephalic furrows.

The *sexual glands*, which alternate with the diverticula of the intestine, become mature in midsummer. The *genital ducts*, when fully formed, open through the muscular layers to the dorsolateral aspects of the body as in related species.

NOTES ON THE REARING OF THE LARVÆ OF POLYGORDIUS APPENDICULATUS AND ON THE OCCURRENCE OF THE ADULT ON THE ATLANTIC COAST OF AMERICA.

R. P. COWLES,
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As the larvæ of *Polygordius* are often found in abundance at Wood's Hole, Newport, Beaufort, and along the coast of Virginia, it seems strange that the adult *Polygordius*, which has been found so often in Europe and which has been so well monographed by Fraipont, should not be taken on the coast of America.

The larvæ of *Polygordius* were quite abundant at Beaufort, N. C., during August of 1902, and through the kindness of the United States Fish Commission, I was able to collect some of these and watch their development in the Commission's new laboratory. It surprised me to find that fully developed swimming larvæ taken at night and measuring slightly less than 1 mm. in length had metamorphosed by the next morning. They were at this time 2 mm. in length, showing an increase of 1 mm. in length.

Fraipont in his monograph on *Polygordius* says that the trunk in *Polygordius appendiculatus* develops inside of the primary cuticle of the swimming larva, becoming folded more and more and that it becomes quite long before it assumes the form which is found in the later stages of development of other species. Examination of the larva which is found at Beaufort shows that this is the case.

In Fig. 1 is shown the swimming larva some time before metamorphosis. The trunk has begun to be folded and the primary cuticle is still seen unbroken along its side. As this larva develops, the trunk becomes folded more and more. This folding of the trunk and the exceptional increase in the length of the latter before the primary cuticle breaks, affords an explanation of the seemingly rapid metamorphosis of the larva into the adult form and also it shows why the young worm immediately after metamorphosis is so much longer than the fully developed larva.

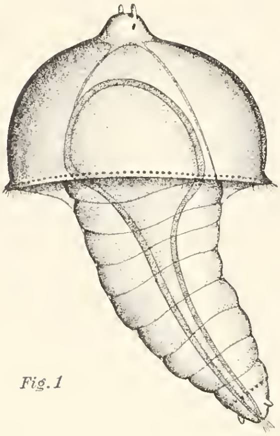

FIG. 1. Larva of *P. appendiculatus*, × 68.

The anal segment is of specific interest in the larva (Fig. 1) which is found at Beaufort, N. C., because it bears the beginnings of two anal filaments which are characteristic of the larva of *Polygordius appendiculatus*. Besides these organs there is on the anal segment a tuft of cilia about the anus. Anterior to the anal filaments there is a ring of glandular papillæ. A pre-anal ring of cilia was not observed.

At the suggestion of Dr. Grave, of the Johns Hopkins University, the young worms, after the completion of metamorphosis, were put into dishes with water and sand rich in diatoms. In order to obtain sufficiently rich cultures of diatoms to afford enough food for the worms, sand was obtained by means of a dredge outside of the harbor and put into aquaria jars with fresh sea water. The jars were then kept in the laboratory near a window until the sand had settled and a rich culture of diatoms had appeared as a brown layer on the top of the sand. This was then drawn off with a pipette and fed to the young worms. Under these conditions they grew rapidly and at the end of six weeks some of them had increased in length to 15 mm. Examination of the contents of the alimentary canal showed that the worms had been feeding on the diatoms.

This method of rearing the young metamorphosed larvæ was first employed by Grave to rear young Echinoderms. By this method also the writer has had good success in rearing the larvæ of *Thalassema* taken from the tow at Beaufort, N. C., and he has half-grown specimens of *Thalassema* which are living and growing under these conditions in the laboratory of Johns Hopkins University.

There is no doubt but what the diatom method is a most useful one in rearing well-developed larvæ taken from the tow.

In Fig. 2 is shown the anal segment of a *Polygordius*, 15 mm. in length, raised by the diatom method. The characteristic anal filaments in this specimen are almost three times the length of the anal segment, as in *P. appendiculatus* as figured and described by Fraipont.

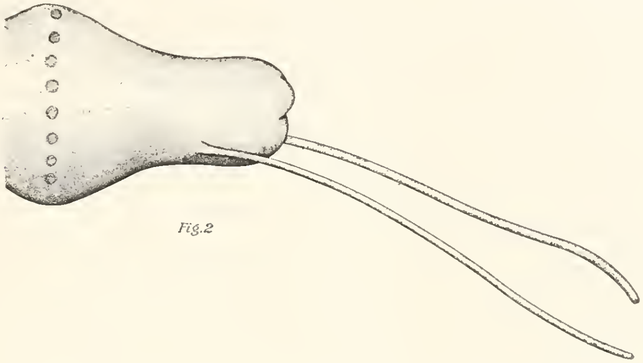

FIG. 2. Anal segment of a specimen of *P. appendiculatus*, × 135.

Sections of the anal filaments show that they are ectoderma. outgrowths surrounded by a cuticle which is continuous with that of the rest of the worm. They are without lumens and the cells seem to be like those of the ectoderm of other parts of the body of the worm. It is possible that the organs have a sensory function, although they are not at all active and are not ciliated. The ring of glandular papillæ present in the larva persists in the adult. In the oldest specimen that the writer has, the superior anal lip is not as distinctly divided into lobes as Fraipont figures, but there is some indication of differentiation into lobes.

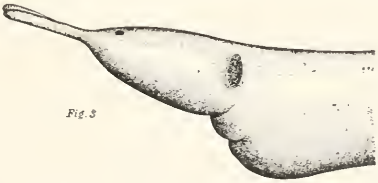

FIG. 3. Cephalic segment of the same specimen as that of Fig. 2, × 135.

The cephalic tentacles (Fig. 3) are seen to be without setæ and longer than in other species, as is the case in *P. appendiculatus*

Sections show that there is no circular muscle layer below the epidermis and also that there is no muscular layer enveloping the alimentary canal. Fraipont observed these facts also.

Although the worms reared by the diatom method had not reached sexual maturity, there can be no doubt but what the form is *P. appendiculatus*, since the two characteristics which distinguish this species from all others are present, namely :

The advanced stage of the development of the trunk before the primary cuticle of the larva is broken and the presence of the two filaments on the anal segment.

There is another *Polygordius* larva which has been taken in the tow at Beaufort, N. C., but its metamorphosis is different from that of *P. appendiculatus* and the anal filaments are not present. Although the writer has not attempted to rear the larva, its metamorphosis and anatomy resemble that of *P. neapolitanus*.

The *Polygordii*, raised by the diatom method, were found to be very active little worms. By means of the glandular papillæ on the anal segments, they were usually found attached to the bottom of the dish, but the rest of the body anterior to this was kept in almost continual motion, waving back and forth and often tying itself into knots.

It is not strange if *Polygordius* has not been found on this coast. In addition to the fact that they are small, thread-like worms living free in the sand, they have the habit of coiling themselves into a minute snake-like coil when disturbed. If they are not more abundant than the most abundant annelids at Beaufort, it would only be by the most painstaking search that they would be found.

ON THE EFFECT OF VARIATIONS IN THE TEMPERATURE UPON THE PROCESS OF ARTIFICIAL PARTHENOGENESIS.

ARTHUR W. GREELEY.

In a previous paper[1] it has been shown that the unfertilized eggs of *Asterias* may be made to develop parthenogenetically by exposing them for a definite length of time to a temperature of 1° C. to 7°C. These experiments were the outcome of others[2] in which it had been demonstrated that the cells of *Spirogyra* and some Protozoa may be made to lose water by lowering the temperature. From these observations on the effect of low temperature on simple animal and plant cells, it is made probable that the artificial development of the *Asterias* egg by means of a lowering of the temperature is brought about by an extraction of water from the protoplasm, just as if the eggs had been placed in a solution of a higher osmotic pressure than that of the sea-water. This latter method has been found to be successful for the *Asterias* as well as the *Arbacia* egg by Dr. C. H. Neilson. Beyond the fact that a low temperature brings about certain changes in the physical condition of the protoplasm that result in a loss of water, nothing is known concerning the action of low temperatures upon the egg.

In the course of experiments on artificial parthenogenesis it has been noted by several observers that the experiment must be performed within certain very narrow limits of temperature. Above or below these limits of temperature, of which 20° C. may be taken as the mean, the experiments have failed with any of the methods heretofore used to obtain artificial parthenogenesis. In order to determine, if possible, the rôle which temperature plays in this process, Dr. Loeb suggested that I repeat some of the well-known experiments in artificial parthenogenesis, test-ing the effect of the solution upon the egg at different temperatures.

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[1] Greeley, *American Journal of Physiology*, VI., 1902, p. 296.

[2] Greeley, *ibid.*, VI., 1901, p. 122.

All the salt solutions used in the experiments on artificial parthenogenesis may be divided into two classes according to their supposed action upon the protoplasm: first, those solutions used at a higher concentration than that of sea-water which produce parthenogenesis through the extraction of water from the protoplasm; and, second, those solutions used at the same or at a less concentration than that of the sea-water which affect the egg through the specific action of ions. In the light of the observations already made on the effect of changes in temperature upon protoplasm, it was a point of considerable interest to determine whether variations in the temperature would affect the action of each of these classes of solutions upon the egg in the same way. Since the speed of a chemical combination varies directly with the temperature, it might be supposed that the specific action of an ion upon the egg would be inhibited or slowed by lowering the temperature, and accelerated by raising the temperature, up to the point where the protoplasm begins to go into heat-rigor. And similarly, since a reduction of the temperature extracts water from the protoplasm, the action of concentrated solutions upon the egg ought to be increased by a lowering of the temperature. Such a disparity between the effect of variations in the temperature upon the chemical and osmotic methods of producing artificial parthenogenesis was, however, not found to hold. A lowering of the temperature, for example, inhibits or slows the action of all solutions upon the egg, whether they are isotonic or hypertonic to sea-water.

The experiments were performed at Woods Hole, during the summer of 1902, with such temperatures as could readily be obtained in the laboratory with the aid of an ordinary hot-air oven and a refrigerator. Four temperatures were used; namely, 30° C., 23° C., 11° C. and 2° C. The solutions to be used in the experiments were prepared and divided among four dishes. The dishes were placed at these four temperatures, and allowed to stand forty-five minutes before the addition of the eggs. The eggs were then distributed among the various dishes at the different temperatures, and after certain intervals of time, were removed to fresh sea-water at the temperature of the room. After removal to the room temperature the eggs were carefully examined from time to time to determine the exact proportion of segmentations and swimming larvæ produced by the same solution at the four temperatures mentioned above. Great care was taken that, with the exception of temperature, all the conditions affecting each lot of eggs might be identical.

The following eggs and methods were used: *Arbacia* eggs, action of concentrated solutions of $MgCl_2$, NaCl, and of sea-water concentrated by evaporation; *Asterias* eggs, action of acids and a concentrated solution of KCl; *Amphitrite* eggs, action of $Ca(NO_3)_2$. Each solution was tried at the four temperatures mentioned above. All these solutions, with the exception of the acids and the $Ca(NO_3)_2$, are of a higher osmotic pressure than the sea-water, and affect the eggs through the extraction of water. In the case of the acids and $Ca(NO_3)_2$, the osmotic pressure remains unchanged, and the fertilization is ascribed to the specific action of the H or Ca ion.

We will consider first the effect of variations of temperature on the process of artificial parthenogenesis when produced by those solutions which extract water from the egg.

Experiment 1.—*Arbacia* eggs were placed in the following solution: 50 c.c. 2½*n* $MgCl_2$ plus 50 c.c. sea-water, and were removed to normal sea-water at intervals of 1, 2, 3½ and 5 hours. The following temperatures were used: 30°, 23°, 11°, 2° C. Only a small proportion of swimming blastulæ were formed at a temperature of 23° C. and about half as many at 11° C. No development took place at 30° C. or 2° C. This was not a successful experiment, as only a small percentage of eggs segmented at all. An increase and a decrease in temperature, to 30° C. and 2° C. served equally well to entirely inhibit segmentation.

Experiment 2.—*Arbacia* eggs were placed in the following solution: 11 c.c. 2½*n* NaCl plus 89 c.c. sea-water. Same temperatures and periods of exposure to the solution as in experiment 1. The results of this experiment will be given in tabulated form as follows:

Periods of Exposure to Solution.

	1 hour.	2 hours.	3½ hours.	5 hours.
30°	0	0	0	0
23°	5	10	0	0
11°	0	5	15	20
2°	0	·5	·5	10

The figures indicate the number of swimming blastulæ formed in 100 eggs. At a temperature of 30° C. no development took place as a result of the NaCl solution, except a few irregular segmentations. At 11° C. a slightly longer residence in the solution over that necessary at the room temperature (23° C.) was required, although at periods of three to five hours a larger percentage of blastulæ were formed than at the room temperature. At a temperature of 2° C. a still longer exposure to the solution was required to produce any effect. At this temperature practically no development took place until the eggs were exposed to the solution for five hours. At 30° C. only a few irregular segmentations occurred as a result of the NaCl solution.

Experiment 3.—*Arbacia* eggs were placed in the following solution: 100 c.c. sea-water concentrated by evaporation to three fourths its volume. This solution has the same relative proportion of ions as normal sea-water, and hence its only effect upon the egg can be the purely physical one of extracting water from it. The same temperature and periods of exposure were used as in experiments 1 and 2. The results will be presented in tabulated form as in experiment 2.

Periods of Exposure to Solution.

	1 hour.	2 hours.	3½ hours.	5 hours.
30°	0	0	0	0
23°	0	4	·5	0
11°	0	15	25	20
2°	0	·5	·4	3

Many more of these eggs segmented at the room temperature (23° C.) than is indicated in the table, but the cells fell apart before complete blastulæ were formed. Practically the same general result is seen as in experiment 2. The optimum period of exposure to the concentrated sea-water becomes increasingly longer as you lower the temperature, but with these longer periods of exposure and low temperatures, a larger percentage of blastulæ are formed than at the temperature of the room. An increase in temperature to 30° C., as in experiment 2, entirely inhibited the development of the egg.

Experiment 4.—*Asterias* eggs were placed in the following solution: 15 c.c. $2\frac{1}{2}n$ KCl plus 85 c.c. sea-water. This method was suggested to me by Dr. C. H. Neilson. Same temperatures were used as in the previous experiments. The eggs were removed from the solution after the following periods: 5 min., 15 min., 30 min., 45 min., 1 hour and 2 hours. The results are tabulated as follows:

PERIODS OF EXPOSURE TO SOLUTION.

	5 min.	15 min.	30 min.	45 min.	1 hour.	2 hours.
30°	1	1	0	0	0	0
23°	20	30	5	0	0	0
11°	0	5	25	20	3	1
2°	0	0	0	.2	1	10

This experiment shows very clearly the inhibiting action of low temperatures on the process of artificial parthenogenesis at short periods of exposure to the solution. The period of exposure necessary to produce development increases steadily as the temperature is lowered, although the optimum period of exposure at any temperature varies greatly according to the maturity of the eggs. The development produced at a temperature of 2° C. after two hours' exposure, was shown not to be due to the low temperature alone by control experiments in which eggs in normal sea-water were kept at this temperature. No development by means of low temperature alone can be obtained, unless the eggs are exposed to the low temperature from three to five hours. At 30° C. again practically no development occurred.

We now turn to the effect of variations in the temperature on the process of artificial parthenogenesis when produced by the action of specific ions.

Experiment 5.—*Asterias* eggs were placed in the following solution: 5 c.c. $n/10$ HCl plus 100 c.c. sea-water. This is a method[1] which has been elaborated by Loeb and Neilson and with favorable lots of eggs yields a very high percentage of development. The same temperatures and periods of exposure were used as in experiment 4. The results are tabulated as follows:

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[1] Loeb, Fischer and Neilson, *Archiv für die ges. Psychologie*, 1901.

Periods of Exposure to Solution.

	5 min.	15 min.	30 min.	45 min.	1 hour.	2 hours.
30°	2	0	0	0	0	0
23°	30	20	1	0	0	0
11°	0	75	75	50	25	0
2°	0	4	15	20	30	2

Practically the same result was obtained as with the KCl solution. The low temperature slows the action of each solution about equally, although the KCl solution extracted water from the egg and the acid solution affects the egg only through the specific action of the H ion. As in the previous experiments, an even larger percentage of larvæ was produced with a low temperature and long exposure, than at the temperature of the room. A temperature of 30° C. had the same inhibiting effect as in the preceding experiments.

Several experiments were performed on the effects of different temperatures upon the process of artificial parthenogenesis in the *Amphitrite* egg when produced by the specific action of the Ca ion. The following solution was used: 4 c.c. *n* $Ca(NO_3)_2$ plus 96 c.c. sea-water. Results were obtained in general similar to those already described for *Arbacia* and *Asterias*, although they were not nearly so uniform. Artificial parthenogenesis occurs at all the four temperatures tried with periods of exposure as short as fifteen minutes, but the optimum period of exposure is much longer at the low temperatures than at the temperature of the room.

In a previous paper[1] I described some experiments on the effects of an increase in temperature alone upon the unfertilized *Asterias* eggs. In no case did I get even a segmentation of the egg by an increase in temperature. These experiments were repeated this summer upon the eggs of *Amphitrite* and *Asterias* with the same result. I modified the experiment in many ways, keeping some of the eggs constantly at a slightly higher temperature than that of the room, while others were returned to normal sea-water after varying periods of exposure to temperatures ranging from 27° C. to 35° C. The protoplasm of the *Asterias* egg

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[1] Greeley, *American Journal of Physiology*, VI., 1902, p. 296.

exists in a very delicate condition of equilibrium as regards its relation to the surrounding temperature. An increase in temperature of only 3° C. over that of the room (24° C. to 27° C.) suffices to liquefy the protoplasm of the egg. Within two hours after the eggs have been exposed to this increase in temperature, the protoplasm loses its granular appearance, becomes clear and homogeneous and flows out, greatly extending the egg membrane. At the same time or before the process of liquefaction has become complete, the nuclear wall breaks down or goes into solution, and the eggs in this stage appear to contain from two to thirty or more nuclear fragments scattered throughout the cell. After six to eight hours' exposure to this increase in temperature, the protoplasm goes into heat-rigor, but beyond the fragmentation of the nucleus, in no case was there even a semblance of segmentation.

This exceedingly delicate condition of equilibrium as regards the physical condition of the protoplasm of the unfertilized *Asterias* egg and its relation to the surrounding temperature, makes it so sensitive to any increase in the temperature, that is seems well-nigh impossible to cause the segmentation of the egg by that means.[1] The subject is by no means closed, however, and further experiments will be performed along this line.

This profound change in the physical condition of the protoplasm as a result of a very slight increase of temperature may explain the fact noted in all the descriptions of the experiments in this paper, that at a temperature of 30° C., artificial parthenogenesis cannot be produced with any of the methods heretofore used. Even at a temperature of 27° C. the protoplasm of the *Asterias* egg becomes completely liquefied, and in this condition no segmentation of the egg can occur.

SUMMARY.

1. The length of exposure to a solution necessary to produce artificial parthenogenesis of the unfertilized eggs of *Asterias* and *Arbacia* varies inversely with the temperature. This applies to

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[1] In a recent paper, however, Delage (*Archives de Zoöl. Exper.*, 1902) describes experiments in which he obtained artificial parthenogenesis of the *Asterias* egg by raising the temperature.

all the solutions used, whether they exert a chemical or an osmotic effect upon the egg. But, at the same time, with lower temperatures and longer periods of exposure to the solution, a larger percentage of larvæ are formed than at the temperature of the room.

2. An increase of temperature to 27° C. liquefies the protoplasm of the *Asterias* egg, and produces a fragmentation of the nucleus. At 30° C. it was found impossible to produce artificial parthenogenesis in *Asterias* or *Arbacia* with any of the solutions used.

EREBOMYRMA, A NEW GENUS OF HYPOGÆIC ANTS FROM TEXAS.[1]

WILLIAM MORTON WHEELER.

The occurrence of a new genus of ants in a country so long known to entomologists as the United States is a matter of surprise when we reflect that the Formicidæ constitute a much smaller family and one much better understood taxonomically than many of those that go to make up the great order of the Hymenoptera, and that, notwithstanding the zeal of collectors, new ant genera are rarely brought to light at the present time even in the most remote and inaccessible regions of the globe.

Early in October Mr. W. H. Long, Jr., kindly sent me from Denton, near the northern boundary of Texas, a number of ants, which, had only the minute yellow workers been present, I should have regarded at first sight as specimens of our common *Solenopsis molesta* Say. But the large males and still larger females in the same vial were so unlike any ants I had ever seen that I undertook a more careful examination of the workers and found them to differ not only from any of the known American genera but also from the Old World genera as well.

In response to a request for data concerning the capture of the specimens, Mr. Long sent me the following: "I have seen this species only once. That was early one morning (I believe it was September 21) after a warm rain the night before. My attention was attracted by an old hen greedily devouring the winged forms as they issued from a small hole in a clear, open space in my back yard. There were no rocks, heaps of earth or surface indications of a nest of any kind. Most of the males and females flew away at once, but here and there I saw a few couples mating near the nest. The diminutive workers fondled and clung to the sexual individuals till the latter escaped into the air. There were many more males than females."

The following is a description of the new genus and species which I take pleasure in dedicating to Mr. Long, as a very slight acknowledgment of his aid in working out the distribution of our Texan Formicidæ.

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[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 45.

EREBOMYRMA gen. nov.[1]

Worker.—Diminutive and monomorphic. Integument yellow, almost without pigment.

Head rather large, suboblong, its posterior border nearly straight, its lateral borders slightly convex. Mandibles rather long, with oblique, 4-toothed blades. Clypeus short, with concave median surface and a pair of teeth on its anterior border. These project downwards rather than forwards and are continued upwards as two distinct ridges on the clypeus. Eyes about one third the length of the head from the insertion of the mandibles, very small, consisting of a single indistinct lens and a few granules of pigment. Ocelli absent. Frontal carinæ short, somewhat further apart than in *Solenopsis*, forming on either side a small lappet covering the insertion of the antenna and then suddenly diverging posteriorly and ending abruptly. Frontal area small, triangular, indistinct. Frontal groove obsolete. Antennal foveæ well-developed. Antennæ 11-jointed; scape of the usual form, first to eighth joint of funiculus together hardly as long as the distinctly 2-jointed club; first funicular joint as long as the four succeeding joints taken together and distinctly broader; joints 2–7 broader than long; eighth joint about as long as broad; basal joint of club about two fifths as long as the terminal joint. Labial palpi 2-jointed, maxillary palpi 1-jointed. Thorax considerably narrower than the head, prothorax with rounded humeri, somewhat flattened above and not separated by a distinct suture from the mesothorax; meso- and metathorax of nearly the same width and distinctly narrower than the prothorax; meso- and epinotum separated by a deep constriction, epinotum armed on either side with a flattened tooth which is hardly longer than broad at its base and continued downwards and backwards as a distinct lamella; dorsal and declivous surfaces of epinotum of about equal length. Petiole in profile much larger than the postpetiole; with a high, rounded node and a slender median tooth on its anterior ventral surface. Seen from above the petiole is more than twice as long as broad, widest behind the middle, slender and subpedunculate in front. Postpetiole seen from above broader behind than in front, campanulate, its posterior edge about twice as broad as the petiole; in profile its dorsal and ventral surfaces are moderately convex. Gaster rather small, narrower than the head, elongate elliptical and somewhat flattened dorsoventrally; anal opening distinctly on the ventral surface, in front of the apex of the gaster. Sting small and apparently vesigial. Legs robust, the femora and tibiæ incrassated, the former towards the middle, the latter towards their distal ends. Middle and hind tibiæ without spurs. Claws simple.

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[1] From Ἔρεβος, Erebos and *μύρμος*, ant; an allusion to the subterranean habits and the gloomy coloring of the males and females.

Female.—Very large as compared with the worker ; deeply colored.

Head subquadrate, convex above ; posterior border slightly concave, sides nearly parallel, posterior angles rounded. Eyes and ocelli rather small, the former round and placed in front of the middle of the head ; median ocellus depressed. Mandibles convex, with oblique 6-toothed blades. clypeus short and broad, somewhat flattened in the middle, the teeth and their longitudinal ridges on the anterior border obsolescent. Frontal carinæ short, rather evenly diverging posteriorly, in front forming a slight fold over the antennal insertion. Frontal area triangular, longer than wide, continued back as a rather deep frontal groove as far as the anterior ocellus, just in front of which it becomes somewhat broader. Antennæ 11-jointed ; scape very short, not reaching the posterior orbit, distinctly incrassated. Funiculus short and compact, with an indistinctly 3-jointed club, the penultimate joint of which is more than half as long as the terminal joint, the antepenultimate nearly half as long as the penultimate and but little thicker than the basal joints of the funiculus. Thorax of the usual structure ; epinotum with a blunt tooth on either side continued downwards to the posterior edge as a low, rounded ridge. Petiole seen from above not broader than long, its base very shortly pedunculate ; anterior declivity long and very convex, at the summit passing abruptly into the very concave posterior declivity, so that the summit of the node forms a trenchant transverse ridge ; lateral surface on either side longitudinally carinate, ventral surface somewhat compressed and produced anteriorly into an acute median tooth like that of the worker. Postpetiole in profile but little smaller than the petiole, and when seen from above but little broader ; its anterior border straight, its anterior angles rounded, its posterior border semicircular and fitting back into a deep semicircular excision of the first gastric segment ; sides of the postpetiole carinate. Gaster large, fully two and one half times as long as broad and but little broader that high. Anal opening and sting inconspicuous, decidedly ventral in position. Legs rather short and weak, the terminal joints of the tarsi more tapering than in the worker. Wings of the usual form, long and well developed. Venation much like that of *Solenopsis*, with well-developed radial, cubital and discoidal cell ; the last larger than in *Solenopsis* and its opposite sides much more nearly parallel ; external branch of cubital vein turning forwards and meeting the costa some distance in front of the tip of the wing ; median vein and internal branch of cubital reaching very nearly to the margin of the wing ; posterior cross-vein short and perpendicular to the median and internal veins. Pterostigma well developed.

Male.—Much larger than the worker but smaller than the female, deeply colored.

Head in proportion to the thorax much larger than in *Solenopsis ;* excluding the eyes distinctly broader than long, rounded behind. Eyes and ocelli very large and prominent. Cheeks much longer than in *Solenopsis.* Mandibles well developed, overlapping, with very oblique 4-toothed blades. Clypeus about as long as broad, very conspicuously convex, hemispherical, its anterior border somewhat truncated, without teeth. There is a small, round, deep pit on either side near the base of the clypeus. Frontal groove and carinæ hardly developed. Antennæ rather short, 13-jointed, of nearly uniform thickness throughout except for the scape and second joint, the former being somewhat thicker, the latter somewhat narrower than the other joints ; scape and joints 3–13 cylindrical, more than twice as long as broad ; second joint about half as long as any of the succeeding joints, of the usual shape and not globose as in *Solenopsis.* Thorax large, with unarmed epinotum or in some specimens with only blunt protuberances in the place of the worker armature. Petiole hardly pedunculate, in profile a little longer than high, its lower surface without a tooth, its node moderate, with the anterior declivity longer and more uniformly sloping than the posterior. Postpetiole shorter than the petiole but twice as broad, campanulate, broadly open behind and conspicuously overlapping the first gastric segment especially on the dorsal side. Gaster elongate-elliptical, rather pointed posteriorly, distinctly compressed dorsoventrally. Genitalia more or less, in some specimens considerably, exserted. Legs rather long and slender. Wings like those of the female.

EREBOMYRMA LONGII sp. nov.

Worker.—(Figs. 1 and 2.) Length 1.5–2.25 mm.

Varying from amber yellow throughout to pale brown ; only the teeth and edges of the mandibles dark brown or black.

Mandibles shining, somewhat striated, with coarse piligerous punctures. Clypeus in the middle between the longitudinal ridges smooth and shining, sides more opaque and rugose. Anterior angles of head and outer portions of antennal foveæ subopaque, traversed by regular and parallel longitudinal rugæ. Frontal area and upper surface of head smooth and shining, covered with rather coarse piligerous punctures. There are a few longitudinal rugæ extending back from the frontal carinæ half way to the posterior border of the head. Sides and ventral surface of head opaque, reticulate-rugose. Antennal scape reaching half way to the poster or angle of the head, slender at the base and somewhat thickened towards the apex. Pro- and mesonotum smooth and shining, with indistinct piligerous punctures. Mesopleuræ and epinotum coarsely and evenly reticulate-rugose, even to the tips of the teeth and the space included between them. Petiole similarly, but somewhat less coarsely, reticulate-rugose, except on the upper surface of the node which is smooth and shining. Postpetiole, gaster, legs and antennæ smooth and shining.

Whole body covered with rather long and abundant pale yellow hairs which on the mandibles, head and thorax arise from the punctures. These hairs are longest on the clypeus and posterior segments of the gaster.

They are conspicuous on the legs and antennæ, especially on the scape and all the joints of the funiculus except the club. On the upper surface of the head the hairs are somewhat more appressed and directed from either side towards the median line which is rather bare. There is no pubescence.

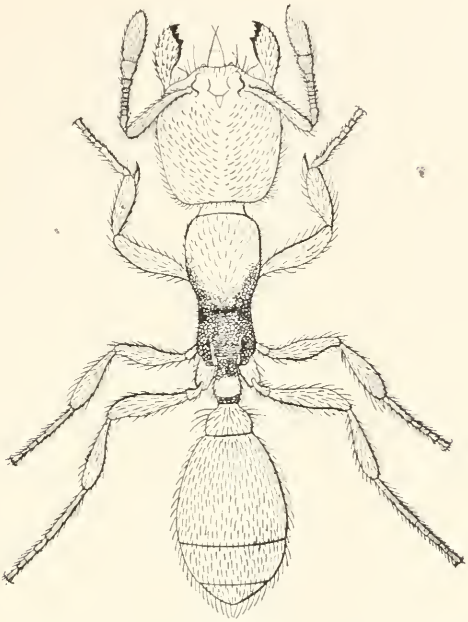

FIG. 1. *Erebomyrma Longii* sp. nov. *Worker.* (Dorsal view.)

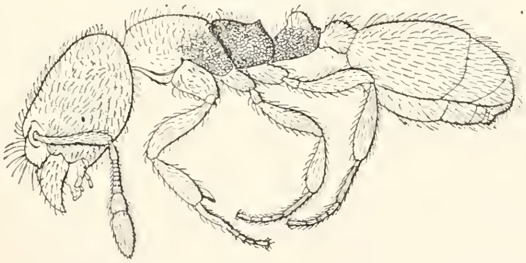

FIG. 2, *Erebomyrma Longii* sp. nov. *Worker.* (Lateral view.)

Female.—(Figs. 3 and 4.) Length 8–8.5 mm.

Black; abdomen, antennæ and legs blood-red; wing insertions, metanotum, lower portions of epinotum, petiole, and postpetiole, the frontal carinæ and lateral portions of clypeus suffused with red; mandibles black, with a broad red band across their apical third; bases of coxæ and middle portions of femora black; wings black, except their apical third which is hyaline; nervures and stigma black.

Mandibles very smooth and shining, covered with coarse piligerous punctures irregularly interspersed with much smaller punctures. Middle portion of clypeus smooth and shining, finely and irregularly punctate; outer portions grossly punctate except laterally where they are very coarsely longitudinally rugose. Frontal area subopaque. Head opaque, very coarsely and evenly longitudinally rugose, the spaces between the rugæ being faintly and confluently foveolate. In the antennal foveæ the rugæ are beautifully concentric, on the front and sides of the head they are longitudinal, but in the region of the ocelli diverge and separate into two systems, passing to the posterior angles of the head. On the occiput the space between the diverging series is filled by a transverse series of rugæ. Antennal scape rugose and very coarsely punctate, or foveolate. Thorax largely opaque, pronotum and neck more densely reticulate and longitudi-

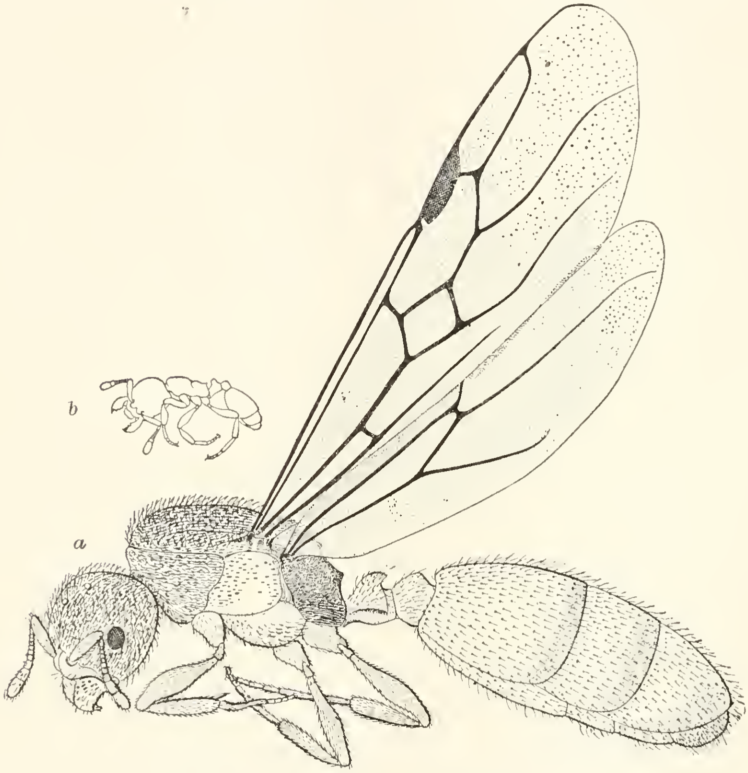

Fig. 3. *a. Erebomyrma Longii* sp. nov. Female. *b.* Worker drawn under the same magnification.

nally rugose than the head; mesonotum subopaque, with a smooth, shining band down the middle and along each parapsidal furrow; with the exception of these regions the whole surface is covered with large elongate-elliptical foveolæ, the spaces between which are more finely punctate and raised into indistinct longitudinal rugæ. Paraptera and scutellum shining, with transversely elliptical foveolæ which are almost absent in the middle of the latter sclerite. Mesopleuræ shining and foveolate like the mesonotum. Surface of metanotum irregularly foveolate and in addition covered with fine, more or less longitudinal rugæ. Epinotum very opaque, densely punctate above and longitudinally rugose below. In the region between the teeth and the ridges running backwards and downwards from them, the surface is crossed by rather coarse transverse rugæ. Convex dorsal surface of petiole very smooth and shining, finely and sparsely punctate and with a few round foveolæ which are most numerous along the posterior edge and the sides of the node; concave posterior dorsal declivity and the whole ventral surface of the petiole opaque, very finely and densely punctate, the former in addition with indistinct rugæ radiating from the posterior edge of the segment. Postpetiole above in the middle shining, with several round foveolæ, which on the sides become prolonged backwards so that the surface has a somewhat grooved appearance; lower surface opaque and densely punctate like the lower surface of the petiole. Gaster shining, the segments smooth and very finely and sparsely punctate at their bases but more opaque and evenly reticulate along their distal borders. In addition to this sculpturing there is a small cluster of impressed reticulations around the insertion of each hair. Legs coarsely punctate-foveolate.

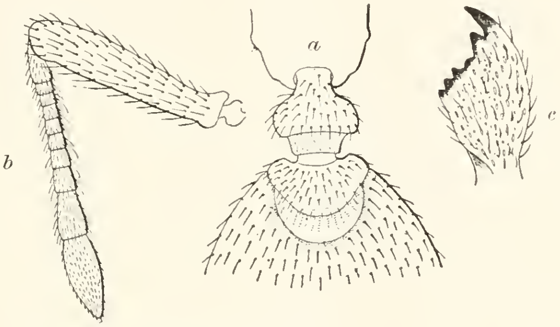

FIG. 4. *Erebomyrma Longii* sp. nov. Female. *a.* pedicel and base of gaster. (dorsal view.) *b.* antenna; *c.* mandible.

Body, except the epinotum, covered with tawny hairs which are relatively shorter than in the worker. On the head, thorax and femora the hairs are erect, on the petiole, postpetiole and gaster suberect, on the antennæ, tibiæ and tarsi shorter and more appressed. Wings covered with minute black hairs.

Male.—(Fig. 5.) Length 5–5.5 mm.

Black; venter and posterior margins of gastric segments fuscous; antennæ whitish but appearing somewhat infuscated on account of a covering of very short black hairs; antennal scape black, second joint paler than the succeeding joints. Mandibles reddish, black only at their bases. Tarsi infuscated from the tip of the first joint. Wings blackened, apical third hyaline; veins and stigma black.

Mandibles longitudinally striated, especially at the base; smooth and shining towards their tips. Clypeus shining in the middle, irregularly and coarsely rugose at its lateral and posterior edges. Head subopaque, with several systems of rather indistinct, parallel rugæ with smooth interrugal spaces; one system runs transversely just behind the clypeus, another on either side from the frontal carina obliquely to the anterior ocellus, where it meets the corresponding series from the other side; another system runs transversely between the two posterior ocelli, while still another is continued downwards from each of these ocelli to the sides and back of the head.

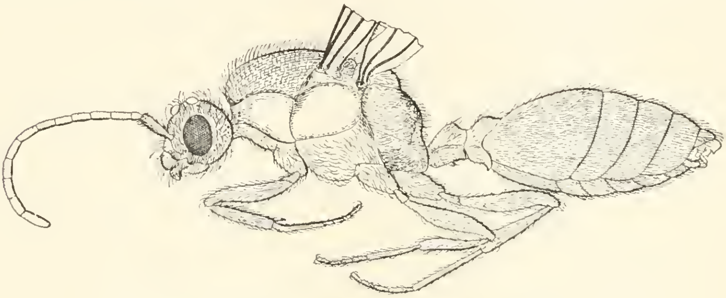

FIG. 5. *Erebomyrma Longii* sp, nov. Male.

Mesonotum subopaque, remainder of thorax smooth and shining except the neck and mesopleuræ which are opaque. Mesonotum with a smooth median band only on its anterior half, the remaining surface more densely covered with elliptical foveolæ than in the female. Metanotum and posterior portion of scutellum with fine parallel transverse rugæ. Mesopleuræ and sides of pronotum sparsely foveolate. Metapleuræ longitudinally rugose. Epinotum almost impunctate. Petiole and postpetiole somewhat roughened and subopaque, node of former smooth and shining, as is also the gaster. The sculpture of the gaster is like that of the female but more indistinct.

Body covered with rather dense, yellowish-gray hairs, which are suberect on the head, thorax and abdomen, but appressed on the legs. There are a few hairs on the shining surface of the epinotum and on the petiolar node. On the antennæ the hairs are microscopic, except on the scape where they are dense and rather conspicuous. Wings covered with minute black hairs.

Described from numerous workers and males and four females from Denton, Denton County, north Texas.

The genus *Erebomyrma* is to be placed in the Myrmicine tribe Solenopsidii, which is known to embrace the following genera: *Solenopsis* Westwood (cosmopolitan); *Diplomorium* Mayr (South Africa); *Aëromyrma* Forel (Madagascar); *Oligomyrmex* Mayr (India, Australia); *Carebara* Smith (Africa, Australasia); *Tranopelta* Mayr (South America); *Lophomyrmex* Emery (India); and *Pheidologeton* Smith (India and Australasia).[1] With the exception of the first and last, these genera are represented each by only one or a few species, and in some cases the sexual forms are imperfectly known. Of *Tranopelta* the workers are unknown, unless the workers from Paraguay mentioned by Mayr (Suedafrikanische Formiciden, 1901, p. 17) as having a distinctly 3-jointed antennal club, 3-jointed maxillary palpi and very small eyes, belong to this genus. In view of these facts a future revision of the tribe Solenopsidii may lead to modifications in the definition of the genera including the one described in this paper. The genera *Aëromyrma*, *Oligomyrmex*, *Pheidologeton* and certain species of *Solenopsis* are characterized by having highly dimorphic workers. In many cases these are connected by a more or less complete series of intermediate forms in the same nest (*e. g.*, *Pheidologeton*, *Solenopsis geminata*). *Erebomyrma* agrees with *Diplomorium*, *Carebara* and most species of *Solenopsis* in having workers of one caste only, and these are extremely diminutive as compared with the males and especially the females. *Erebomyrma* is evidently most closely related to *Solenopsis*, *Diplomorium* and *Aëromyrma*, but differs from the two former in having the epinotum armed and from the latter in having 11- instead of 10-jointed antennæ, and in the absence of any dimorphism in the workers. The latter character is variable, however, since in one genus (*Solenopsis*) it is absent in most of the species, though highly developed in others. The female of *Erebomyrma* is colored and sculptured much like the female of the African *Carebara vidua* Smith, while the male seems to have many points in common with the male of *Tranopelta*. It is probable that *Erebomyrma* is not a monotypic genus but comprises also several South American species. At least Professor Emery writes me that he has in his collection two species which seem to belong to the new genus. Both of these are represented by female specimens only. One is from Bolivia, the other from Rio de Janeiro.

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[1] The genus *Melissotarsus* Emery, formerly supposed to belong to this group of genera, is now placed among the Ponerinæ by Emery.

Comparatively little is known concerning the ethology of the Solenopsidii apart from the genus *Solenopsis*, which has many representatives in Europe and North America. The majority of the species belonging to the tribe, if we except *Pheidologeton* and the larger forms of *Solenopsis*, like *S. geminata*, appear to have certain common ethological traits of more than usual interest. These characters, which were first appreciated by Forel, and constitute one of the many fine discoveries of that able naturalist, are the following:

1. The males and especially the females of the smaller species of *Solenopsis*, the species of *Aëromyrma*, *Carebara*, *Oligomyrmex*, and presumably also of *Tranopelta*, are of very large size compared with the workers. The same is also true of *Pheidologeton* and the polymorphic species of *Solenopsis* when the sexual forms are compared with the most diminutive caste of workers. The relative dimensions of the queens differ, however, considerably in the different genera. Thus in our common North American *Solenopsis molesta* the workers measure 1.5 mm. in length, the females 4.5–5 mm.; while in *Carebara vidua* the worker is hardly larger than that of *S. molesta* (1.5–2 mm.) whereas the female is of gigantic dimensions (23 mm.). The dimensions of *Erebomyrma Longii* lie between these extremes, though much nearer to those of *S. molesta*. The worker is 1.5–2.25 mm. long, the female 8–8.5 mm. If we cube the dimensions in these three species and make due allowance for the fact that the body of the female ant is in each case proportionally much thicker than that of the worker, we have the following roughly approximate ratios between the volumes of the workers and females:

Solenopsis molesta,	1 : 20.
Erebomyrma Longii,	1 : 150
Carebara vidua,	1 : 2000.

These are rather extraordinary dimensions for queens as compared with workers, especially when we reflect that they represent the sterile and fertile extremes of the same sex.[1]

2. The workers of the species in question all have a pale, etiolated appearance, being uniformly yellow or light brown in color, while the huge males and females are deeply and often conspicuously colored. This is noticeably the case with *Carebara* and *Erebomyrma*.

3. The eyes of the workers are vestigial or quite absent (*Carebara*), in marked contrast with the well-developed eyes and ocelli of the males and females.

4. As we should naturally infer from the characters enumerated under 2 and 3, these ants are hypogæic or subterranean, *i. e.*, rarely or never coming to the surface except during the nuptial flight of the deeply colored sexual forms.

5. It is clear that the diminutive workers must be able to obtain large quantities of food, or they could never raise so many and such enormous males and females. From this, again, we may infer that the species prey on other ants or termites, and this inference is supported by observation in all cases where it has been possible to study these ants in their nests. The European *Solenopsis fugax*, the North African *S. latro*, the North American *S. molesta* and *S. texana*, and probably many other small species of the genus, live in the nests of larger ants belonging to different genera and species (*Formica*, *Aphænogaster*, etc.). Here they inhabit small chambers in the walls separating the galleries of the larger species and, escaping notice, probably on account of their minute size and neutral nest-odor, prey upon the helpless and well-fed larvæ and pupæ of their hosts. This mode of life has been recently called "*lestobiosis*" by Forel, who has directed attention to similar habits in *Aëromyrma* and *Carebara*. Sikora found *Aëromyrma Nosindambo* Forel, of Madagascar, as a regular inhabitant in the earthen nests of termites, and Haviland

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[1] Other cases comparable to the extreme disproportions of the female and worker *Carebara* are certainly rare but they occur nevertheless in *Pheidologeton* and in *Atta* (*s. str.*). The minimum workers of the Texan *Atta fervens* Say are barely 2 mm. long, whereas the queens measure fully 17 mm. Among some specimens of the Bengalese *Pheidologeton ocellifer* Smith given me by Professor Forel, I find diminutive workers only 2.25 mm. long and a queen of 16 mm. The relative differences in volume in these cases can be approximately computed without difficulty

found *Carebara vidua* of South Africa also living in lestobiosis in the clay nests of termites (*Termes natalensis*). A consideration of these facts and the taxonomic affinities of *Erebomyrma Longii* led me to surmise that this species too must be lestobiotic, in all probability not with other ants but with some of our Texan termites. That the species is hypogæic would seem to be perfectly clear from Mr. Long's statements quoted in the opening paragraphs of this paper. A second letter, in response to a request urging him to search for termite nests on the spot where he found the *Erebomyrma*, tends to confirm my suspicions of its lestobiotic habits. Mr. Long says: "There seems to be a great number of termites in this vicinity, as I found the sexual forms issuing in great numbers from many holes in my back yard, just like the ants of the new genus which I sent you. Several of these holes were very close to the spot where the ants were captured." During the coming year Mr. Long will endeavor to obtain more definite data concerning the habits of the interesting ant which he has brought to my notice.

Austin, Texas,
November 29, 1902.

Vol. IV. *March, 1903.* *No. 4*

BIOLOGICAL BULLETIN.

DIMORPHIC QUEENS IN AN AMERICAN ANT (LASIUS LATIPES WALSH).[1]

W. M. WHEELER AND J. F. McCLENDON.

On the afternoon of September 17 of the current year the senior author had occasion to witness the nuptial flights of several species of *Lasius* in an open wood near Rockford, Illinois. These flights occurred almost simultaneously from mound nests of *Lasius niger* var. *americanus*, *L. claviger* and *L. latipes.* The first species is ubiquitous in all open country in the Northern States, especially where the soil is sandy or loamy. Owing to the dingy color of the workers, males and females, and the relatively small size of the colonies, the nuptial flight of this species offers nothing of special interest or beauty. It is quite otherwise with some of the yellow *Lasius*, of which at least eight species are known to occur in the United States, namely: *L. aphidicola* Walsh, *speculiventris* Emery, *brevicornis* Emery, *myops* Forel, *interjectus* Mayr, *claviger* Roger, *latipes* Walsh, and *Murphyi* Forel. The last is known only from North Carolina and Colorado. *L. interjectus*, *aphidicola*, *claviger* and *latipes* build large mound nests, often a foot or more in diameter and several inches high, either in open grassy places or about the bases of rotting stumps. These mounds are shot through with living grass and covered with little openings for the ingress and egress of the ants. *L. latipes* in some localities prefers to build its nests under rather large stones. This is the case at Colebrook, Connecticut, for example. Unlike *L. niger* and its varieties the yellow species of the genus appear to be nocturnal in their habits and *L. myops* largely subterranean. At any rate the workers of these various species are not seen to leave the nests in the day-time except during the nuptial flight of the males and virgin females.

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[1] Contributions from the Zoölogical Laboratory of the University of Texas. No. 46.

This flight, especially in the case of *L. latipes*, presents a beautiful spectacle. At the moment when the great swarming impulse seizes the colony, the shining amber-yellow workers, together with the hosts of sable males and large tawny-red females, break in a flood from the main openings of the nest.

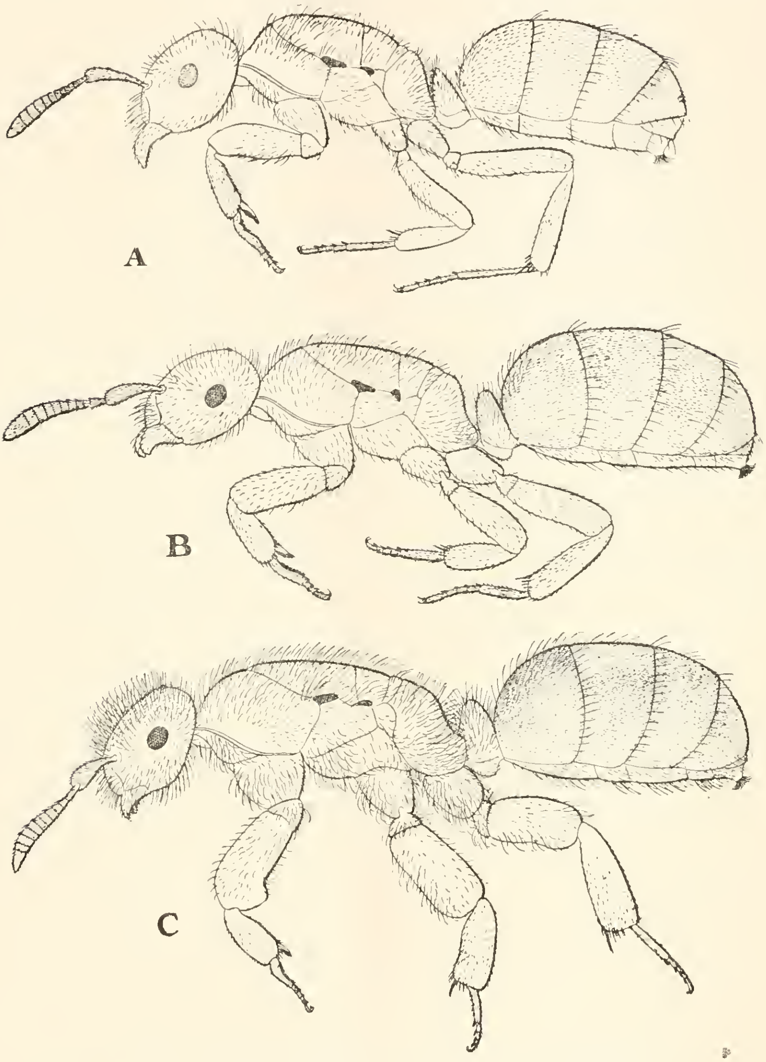

FIG. 1. *A*, *Lasius claviger* Roger ♀; *B*, *Lasius latipes* Walsh α-♀; *C*, *L. latipes* β-♀. The wings are omitted.

The winged forms hasten up the slender grass-blades on which they rock for a few moments, while filling their tracheæ with the pure air of the upper world, then one by one spread their glittering wings and soar into the air like sparks rising from a fire.

While watching a colony during this interesting culmination of its annual development, the senior author noticed females of two different kinds issuing in numbers from the same openings of the grass-covered mound. The majority of these females were the remarkably pilose individuals, of a rich fulvous red, with extremely broad and flat legs and abnormally short, feeble tarsi, which have always been regarded as the true females of *L. latipes*. Among these, however, there were several hundred females which were perceptibly smaller, of a deep brown color, much less pilose, with only moderately broadened and compressed legs and with much longer tarsi. Both forms mingled with the workers and males and took flight together within the same half hour. Although the unusual character of this observation was fully appreciated at the time, circumstances made it impossible to excavate the nest and search its penetralia for the mothers of these very different virgin females. It seemed best to leave the nest for careful study at some future time and to collect a large number of the workers, males and females at the surface.

In this paper we will designate as the β-female the highly aberrant form (Fig. 1, *C*) with the excessively flattened legs, *i. e.*, the form which has hitherto passed as the true and only female of *latipes;* the other (Fig. 1, *B*) we will call the α-female. These designations will suffice for present purposes and will leave the facts uncolored by the conjectural meaning of this singular dimorphism.

A few days after the above recorded observations were made the senior author returned to Texas, and soon afterwards, with the aid of the junior author, undertook an examination of all the material of *L. latipes* collected during three consecutive summers in three different localities. This was easily possible because the specimens from different nests had been kept by themselves in separate vials of alcohol. There were, in all, collections from ten separate nests, as recorded with the date of capture and the personnel of each colony in the following table:

Nest No. 1. Woods Hole, Mass., Aug., 1900. ☿, ♂, β-♀.
Nest No. 2. Woods Hole, Mass., Aug., 1900. ☿, β-♀.

Nest No. 3. Colebrook, Conn., Aug. 19, 1901. ☿, β-♀.
Nest No. 4. Colebrook, Conn., July 21, 1901. ☿, β-♀.
Nest No. 5. Colebrook, Conn., Aug., 1901. ☿, ♂, α-♀, β-♀.
Nest No. 6. Colebrook, Conn., Aug. 12, 1901. ☿, ♂, α-♀.
Nest No. 7. Rockford, Ill., July 6, 1902. ☿.
Nest No. 8. Rockford, Ill., Aug. 15, 1902. ☿, ♂.
Nest No. 9. Rockford, Ill., Aug. 20, 1902. ☿, ♂.
Nest No. 10. Rockford, Ill., Sept. 17, 1902. ☿, ♂, α-♀, β-♀.

Nest No. 1 was collected by Miss Adele M. Fielde. No. 2 was an artificial nest seen in one of the laboratories at Woods Hole. It contained many workers and a single deälated female of the β-type. Of the ten nests in the table it will be seen that three contained workers only, or workers and males. These may be disregarded as having no bearing on the subject under consideration. Of the seven nests containing females, four contained β-females only; two contained both α- and β-females, and one contained α-females only. Thus it is seen that the α-female, which has not been observed hitherto, occurred in three out of seven nests, and in two very widely separated localities. This is significant in view of the fact that *L. latipes* is not nearly so common a species as *L. claviger*, *interjectus*, *myops* or *aphidicola*.

Examination of a considerable number of females of both types failed to disclose any forms intermediate in structure or pilosity. In nest No. 5, however, all the β-females had the same deep brown color as the α-females of the same nest. There were often considerable individual variations in the venation of the wings, but these variations occurred in both types indiscriminately. The types were not connected by intermediate forms and were indistinguishable from each other by any characters in the shape, color, or venation of the wings, so that these organs could be omitted in the figures and in the comparative statements to be given below.

A study of the descriptions of *L. latipes* by previous writers shows very clearly that the β-female has played a very important rôle in the recognition of the species, because it differs so markedly in structure and pilosity from the females of any of the known members of the genus. The original description by Walsh ('62, p. 311) is so brief and inadequate that it would have been con-

	L. claviger. ♀*	L. latipes. α-♀	L. latipes. β-♀
Length of thorax	2,517	3,390	3,487
Length of gaster	3,055	3,681	3,487
Length of petiole	285	303	392
Height of petiole	872	926	943
Length of antennal scape	926	890	854
Apical breadth of antennal scape	178	230	267
Length of funiculus	1,566	1,513	1,370
Breadth of funiculus	214	231	249
Length of fore femur	1,264	1,264	1,335
Length of middle femur	1.193	1,175	1,282
Length of hind femur	1,356	1,388	1,460
Breadth of fore femur	427	498	659
Breadth of middle femur	303	427	605
Breadth of hind femur	356	463	623
Length of fore tibia	1,068	1,015	979
Length of middle tibia	1,015	1.050	1,086
Length of hind tibia	1,513	1,513	1,442
Breadth of fore tibia	320	356	445
Breadth of middle tibia	267	338	427
Breadth of hind tibia	267	356	481
Length of fore spur (strigil)	338	320	231
Length of middle spur	231.	249	249
Length of hind spur	267	267	267
Length of whole fore tarsus	1,015	905	729
Length of first fore tarsal joint	570	516	374
Length of second fore tarsal joint	89	89	89
Length of third fore tarsal joint	89	71	71
Length of fourth fore tarsal joint	89	71	53
Length of fifth fore tarsal joint	178	160	142
Length of whole middle tarsus	1,175	961	818
Length of first middle tarsal joint	605	534	409
Length of second middle tarsal joint	125	107	89
Length of third middle tarsal joint	107	89	89
Length of fourth middle tarsal joint	107	89	71
Length of fifth middle tarsal joint	231	142	160
Length of whole hind tarsus	1,691	1,406	997
Length of first hind tarsal joint	979	783	570
Length of second hind tarsal joint	196	160	107
Length of third hind tarsal joint	142	125	89
Length of fourth hind tarsal joint	107	89	71
Length of fifth hind tarsal joint	267	249	160

signed to the limbo of useless specific diagnoses except for the mention of the extraordinarily flattened legs in the female, a character which is, moreover, emphasized in the specific name. Walsh had only two specimens of the β-female. The locality of the types is not given, but was probably Rock Island, Illinois. It was the flattening of the legs of *L. claviger*, a trait still more pronounced in *latipes* and visible also in *interjectus* and the more recently discovered *Murphyi* that led Mayr ('62, p. 51) to separate these forms as the genus *Acanthomyops*. Later he reduced this genus to subgeneric rank under *Lasius*, where it is still used to include those species which have 3- instead of 6-jointed maxillary palpi. Mayr redescribed ('66, p. 889) the β-female from a defective specimen from Wisconsin, and says that he was at first tempted to place it in a new genus on account of its remarkable appearance. That he refrained from doing this is evidence of his keen taxonomic insight. Later writers, like Emery ('93, p. 638), have included the β-female in the table of *Lasius* species as distinguishable from all other females by having "the hind tarsus shorter than the much flattened tibia." The discovery of the α-female, which has the hind tarsus longer and the tibia much less dilated, makes it more difficult to recognize the species. This has induced us to make a closer study of *L. latipes* and of the allied *claviger* in all the sexual phases.

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* The dimensions are in micra.

Comparison shows that the α-female is almost intermediate between the β-female and the female of *claviger*. This is clearly shown in the figures, in the table of measurements on p. 153, drawn up by the junior author, and in the two-column statement of the principal differences between the α- and β-females, as compared with the female of *L. claviger* as a standard:

α-female of Lasius latipes.	β-female of Lasius latipes.
1. Dark brown, like *L. claviger* ♀.	1. Fulvous red, in one nest (No. 5) dark brown like the α-female.
2. A little more pilose and pubescent than *L. claviger* ♀.	2. Much more pilose and pubescent.
3. A little larger.	3. Considerably larger and longer.
4. Thorax longer in proportion to the gaster.	4. Thorax much longer in proportion to the gaster.
5. Mesonotum and scutellum as in *claviger* ♀.	5. Mesonotum and scutellum flatter.
6. Petiole thicker, higher and more rounded above than in *claviger* ♀.	6. Petiole considerably thicker, higher and more rounded above.
7. Mandibles similar to those of *claviger* ♀.	7. Mandibles with fewer teeth than in *claviger* ♀ (Fig. 3 *C*).

8. Antennal scape and funiculus shorter and broader. (Fig. 2, *B*.)	8. Antennal scape and funiculus still shorter and broader. (Fig. 2, *C*).

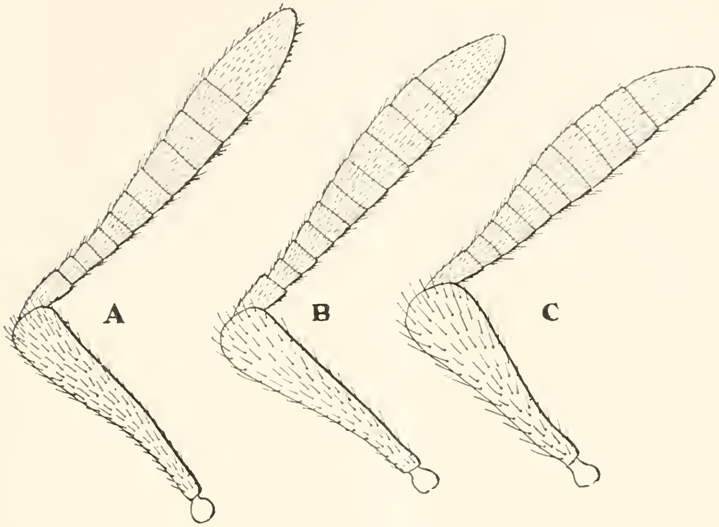

FIG. 2. *A*, Antenna of *Lasius claviger* ♀; *B*, of *L. latipes* α-♀; *C*, of β-♀.

9. Trochanters, femora and tibiæ broader and more flattened.	9. These joints extraordinarily flattened and dilated.
10. Strigil a little smaller.	10. Strigil much smaller.

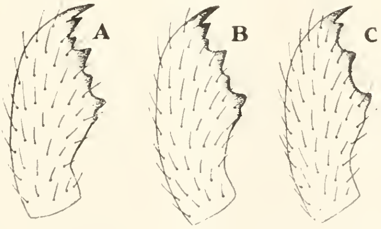

FIG. 3. *A*, Mandible of *Lasius claviger* ♀; *B*, of *L. latipes* α-♀; *C*, of β-♀.

11. All the tarsi a little more tapering.	11. Tarsi rapidly tapering.
12. Middle and hind tarsi nearly as long as the tibiæ.	12. Middle and hind tarsi much shorter than the tibiæ.

Turning now to a comparison of the two species, *latipes* and *claviger*, as exhibited by the workers and males, we find but few points of difference, so that we are compelled to regard the two species as very closely related to each other. The worker *latipes* has a thicker petiole, which is distinctly blunt and rounded above, and the hairs are somewhat more abundant and evenly distributed on the dorsal surface of the gaster. In the worker *claviger*, on the other hand, the petiole is thinner anteroposteriorly and sharply cuneate above when seen in profile, and the gaster is less uniformly hairy and somewhat more shining. The males of the two species differ much as do the workers in the shape of the petiole. Moreover the male *claviger* is decidedly larger, more robust and blacker than the male of *latipes*. These differences, especially in the males, are easily appreciated in the living specimens when they are seen in numbers, but are necessarily more obscure in dried cabinet specimens.

From these comparatively slight differences between the males and workers of the two species we should naturally expect to find a corresponding similarity in the females. It is quite obvious that the α-female is the very form which satisfies this requirement, whereas the β-female presents extreme characters which make it appear like a decided sport or aberration from the normal type of *Lasius* female. It would seem, therefore, that the β-female is the one for which we are most in need of an explanation, although it is connected with the females of normal form by a rather complete series of gradations, *i. e.*, through the females of the following species, beginning with the most extreme form: *L. Murphyi*, α-female of *latipes*, *claviger*, *interjectus*. The remarkable configuration of the legs and antennæ, the color and pilosity of the β-female all suggest some peculiarity of habit or habitat the nature of which remains to be determined by further observation and experiment.

We come now to the important question: What is the meaning of this dimorphism in the females of *L. latipes?* From the fragmentary data at our command it would seem that four different hypotheses might be advanced to explain this peculiar phenomenon:

1. It may be suggested that the α- and β-females really belong to two distinct species. According to this view the α-female might be regarded as the true queen of *latipes*, whereas the

β-form would represent the queen of some inquiline or symbiotic species. Although this explanation is readily suggested by the well-known cases of dulosis and xenobiosis in ants, we are, nevertheless, bound to reject it for the following reasons: Though the β-females were taken in several nests and, in one case, were seen to celebrate their nuptial flight at the very same time as the α-females, no males or workers which could represent any species except *latipes* were to be found in the nests. The same argument would hold *mutatis mutandis*, were we to consider the β-form as the only true female of *latipes*. The workers and males of all the known North American Lasii have been accounted for, and there is still a female form left over, so that there is no species known that could be enslaved by, or live as an inquiline with, *L. latipes*. We should have to suppose that the inquiline species was represented by females only, and this is most improbable. Finally, the deep coloration above noted as occurring in the β-females of nest No. 5 would indicate that both the α- and β-females belong to the same species. We believe, therefore, that this hypothesis may be safely rejected.

2. It may be suggested that the α-female is the normal female of *latipes*, whereas the β-females are diseased forms — individuals afflicted with some strange emmet elephantiasis or acromegaly! But even apart from the very frequent occurrence and uniform development of the β-females, dissection shows that such a view cannot be seriously entertained. Their internal structure is in no respect abnormal. The fat body is well developed and the ovaries are in the same stage and have the same normal structure as the ovaries of the α-females. If anything, the β-females are more vigorous, somewhat larger and supplied with more fatty tissue (even in the distal lobes of the large fore femora!) than the α-females. In a word, the β-females are somewhat above normal, while the α-females, so far as we are able to judge, are quite normal. Hence this hypothesis, also, may be safely rejected.

3. The dimorphism may be regarded as the result of hybridism between *L. claviger* and *L. latipes*. This view is supported by the following considerations:

(*a*) Both species occur in the very same localities, and *latipes* is much rarer than *claviger*. Hence the queens of the latter may find cross-fertilization by males of their own species from other nests very difficult and fertilization by males of *claviger* a relatively easy matter.

(*b*) The nuptial flights of the two species may occur simultaneously. In fact, the senior author witnessed a flight of *claviger* from a nest not twenty feet away from the *latipes* nest and at the very same time (3.30 P. M.) as the above-described flight of the latter species. And it may also be stated that both these nests were large and must therefore have existed side by side for some years. We could suppose that a β-female of *latipes* in some previous year had been fertilized during her nuptial flight by a male *claviger* and had returned into the parental nest to give birth to the α-females which celebrated their nuptial flight on the 17th of September, 1902.

(*c*) This view is also supported by the fact that the α-female is so clearly intermediate in nearly all its characters between the female *claviger* and the β-female, as has been shown in the above tables.

The arguments that can be brought to bear against the hypothesis are the following:

(*a*) We have failed to find any hybrid workers in the nests containing the α- and β-females. This should be the case unless we suppose that all the hybridized β-females produced only queens.[1] But it must be borne in mind that the hybrid between the worker *claviger* and worker *latipes* would differ presumably from the parent species only in intermediate pilosity and in having a petiole intermediate in shape. Such differences would not be easily detected, as anybody will confess who has examined a large series of workers of the two species. The workers are of small size and the petiole is sometimes decidedly variable even within the limits of the same species of *Lasius*.

(*b*) It is improbable that hybridization could occur so frequently in a state of nature as appears to be indicated by the high percentage of nests containing α-females, and their occurrence in such widely separated localities. If we are really confronted by a case of hybridism we are almost compelled to believe that the *α*-female must be sterile, notwithstanding her well-developed ovaries, or the two species would long since have merged into one.[1]

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[1] Obviously the male offspring of the hybridized queen would not be affected, since they arise from unfertilized eggs.

(*c*) It seems improbable that such an aberrant creature as the *β*-female would mate with the male of another species, but this argument loses much of its force when we stop to reflect that the *claviger* male is very similar to the *latipes* male even in the structure of its genitalia.[2]

4. We may suppose that we are dealing with a true case of dimorphism in the female sex. On first thought this seems improbable because dimorphic queens, in the strict sense of the term, are unknown among ants. But when we stop to consider that the social bees and wasps exhibit an essentially similar dimorphism, except that one of the two winged forms, the worker, is sterile (and this may also be the case with the *α*-female of *L. latipes !*) there is nothing preposterous in this view. Moreover, in ants the wingless workers have themselves in many species become dimorphic, developing soldier and typical worker forms, either perfectly distinct from each other or connected by a series of intermediates. Why, then, may we not expect the winged queens in some cases to exhibit dimorphism among themselves, especially when dimorphism "runs in the blood," so to speak, of all the social Hymenoptera? And why may not *L. latipes* be such a species in which the old and deeply-rooted tendency is breaking out in a novel form? This would at least complete the theoretical possibilities in female ants as represented in the following diagram:

Primitive female.

Worker, or sterile female. Queen, or fertile female.

Worker minor. Worker major, or soldier. α-female. β-female.

It thus appears that of the four hypotheses, two may be rejected as too improbable to be entertained, and that the true meaning of the dimorphism of the females of *L. latipes* is to be sought in the direction of hybridism or of dimorphism *sensu stricto.* Only further observation and especially experiment can enable us to decide which of these interesting alternatives confronts us.[1]

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[1] Unless, indeed, the Mendelian law be supposed to operate with unprecedented clearness in this particular case.

[2] In this connection, however, it is interesting to note that Marchal ('96, p. 45) failed to induce mating between two very closely allied species of wasp (*Vespa germanica* and *V. vulgaris*).

For the present we incline to the belief that the α- and β-females of *latipes* represent true dimorphic forms, and see in this condition an interesting repetition of what may have led to the differentiation of the primitive winged female ant into workers and queens. It is granted on all sides that insects like the ants, social wasps and bees, which present three sexual phases, viz., males, queens and workers, are to be derived from forms with only a single female form. In the bees and wasps there can be no question that this original female form was winged like the male, and we should expect this to be the case also with the ants, but so eminent a myrmecologist as Professor Emery takes quite a different view of the matter ('95*b*, p. 775). He says: "If the above considered derivation of ants from Mutillid-like Hymenoptera be granted, we must suppose, furthermore, that in primitive ants, as in the Mutillids, the males were winged, but the females wingless, and that the latter subsequently reacquired wings. This supposition is upheld by the fact that wingless females are most commonly met with among the Dorylinæ and Ponerinæ, *i. e.*, in those very groups of ants which are the most primitive, more rarely among the Myrmicinæ, and most rarely, and, so far as I am aware, only as individual anomalies, in the Dolichoderinæ and Camponotinæ. The frequency of occurrence of wingless females is, therefore, inversely as the phyletic stage of development of the different groups of ants.

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[1] In the Lepidoptera and Hymenoptera, if we except a few cases like the Torymid Chalcididæ, the female sex seems to be more prone to dimorphism than the male. In Diptera the few recorded cases of dimorphism occur in males; *e. g.*, in the Brazilian *Curupira torrentium* (Fritz Mueller, '81; Osten Sacken, '95) and the North American Syrphid *Mallota cimbiciformis* (Williston, '86). Among the Coleoptera *Dytiscus* presents dimorphism in the females, while some of the Anthribidæ are said to show it in the males. The dimorphism seen in the "high" and "low" males of the Scarabæidæ among the Coleoptera and the "high" and "low" male Dermaptera (*Forficula auricularia*) observed by Bateson ('94, pp. 40–42), resembles that of the female *Lasius latipes* in being a normal and excess development of the individuals of the same sex. In the latter case, however, the two forms are not connected by intermediate variations.

"Not only is the normal occurrence of wingless females among existing species evidence of a similar condition among the primitive ants, but it also furnishes the most natural explanation of the origin of the wingless workers. I surmise that *the ancestral ants constituted small societies of wingless females, among which sterile individuals were subsequently differentiated as workers.* The wings, so readily deciduous in the queens of existing ants, were newly acquired from rudiments still persisting in the ontogeny, by a process of reversion to the winged ancestors."

We are unable to assent to this view, for the following reasons:

1. While there is no end of evidence to show that the most diverse insects have lost their wings during phylogeny, there is not, to our knowledge, a single insect which can be satisfactorily shown to have reacquired these organs. At any rate the losing of wings is a much easier process than their acquisition.[1] Emery's hypothesis postulates a winged condition in both sexes of the ancestors of Mutillidæ, a loss of the wings in the females of the Multillid-like ancestors of ants, a persistence of this primitive condition by inheritance in the ancestral Formicidæ and a comparatively recent reacquisition of wings in the females of all except the Dorylinæ and the few Ponerine genera which have wingless females (*Leptogenys*, *Acanthostichus*). This would seem to be a needless complication of matters, apart from the fact that it is venturesome to invoke the obscure principle of reversion to account for the reacquisition of organs.

2. Existing wasps and bees certainly show the possibility of differentition into workers and queens prior to the loss of wings.

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[1] This is an interesting case of a principle to which Headley ('01, pp. 100, 101) has recently called attention: "The sudden loss of horns brings out a point to which, I think, attention has never been directed in discussions on pammixis. The evolution of new characters is a gradual process requiring ages of time. Geology shows that the stag's antlers have grown step by step from small beginnings. But they might be completely lost in a single generation. The horns of cattle, though less magnificent, are none the less the slow product of ages of unintermitted selection. But by a sudden freak they disappear utterly in an individual here and there, or leave only a dangling vestige attached to the skin.

"Those evolutionists who love symmetrical theories, mapped out regardless of observed facts, imagine a process of retrogression by which all the stages are retraced in ordered succession. What actually happens is usually very different. An elaborate organ is suddenly much reduced and mutilated or suddenly disappears altogether."

And there is no strong evidence to show that this condition did not exist in the ancestral ants, for the Dorylinæ are hardly in the direct line of Formicid descent, and the Ponerinæ, though very primitive, still show the differentiation into winged queens and wingless workers in some of their most generalized genera (*Cerapachys*, *Sysphincta*, *Proceratium*, etc.).

3. That the most natural way of accounting for the wingless workers is through loss of the organs of flight in one of the two winged female forms, is also indicated by the phenomena of ergatomorphism among male ants. It is known that in a few sporadic species belonging to several genera the males are wingless and have assumed a worker-like form, especially in the development of the thorax. These species are, *Anergates atratulus* (Schenck '52), *Formicoxenus nitidulus* (Adlerz '84), *Cardiocondyla Stambuloffii* (Forel '92), *Ponera punctatissima* (Emery '95*a*) and *P. ergatandria* (Forel '93). This same reduction of the wings is shown in a more or less advanced condition in some male Mutillidae. All these cases are most naturally explained by loss of the organs of flight, and we are justified in adopting the same explanation to account for the wingless condition of the workers. Our view of the matter, therefore, would differ from Emery's in assuming that in the ancestors of the ants all three forms, workers, queens and males, were alike winged, and that the workers lost their wings either suddenly in accordance with Headley's principle, or concomitantly with the atrophy of the ovaries and the assumption of the other worker characters. Thus it would be the workers that have lost their wings and the queens have not reacquired, but retained these organs which came to them as the common heritage of all the Pterygote insects.

BIBLIOGRAPHY.

Adlerz, G.

'84 Myrmecologiska Studier. 1 *Formicoxenus nitidulus* Nyl. Oefversigt. af Kongl. Vetensk.-Akad. Foerhandl., 1884, No. 8, pp. 43–64, Taf. XXVII., XXVIII.

Bateson, W.

'94 Materials for the Study of Variation treated with Special Regard to Discontinuity in the Origin of Species. London, Macmillan & Co., 1894.

Emery, C.

'93 Beitraege zur Kenntnis der Nordamerikanischen Ameisenfauna. Zool. Jahrb. Abth. f. Syst., Bd. VII., 1893, pp. 633–682, Taf. XXII.

Emery, C.

'95a Sopra Alcune Formiche della Fauna Mediterranea. Mem. R. Accad. Scienze. Sess. 21, Apr., 1895, pp. 291–307, 1 Tab.

Emery, C.

'95b Die Gattung Dorylus Fab. und die systematische Eintheilung der Formiciden. Zool. Jahrb. Abth. f. Syst., Bd. VIII., pp. 685–778, Taf. XIV–XVII., text-figs.

Forel, A.

'92 Le mâle des Cardiocondyla et la Reproduction Consanguine Perpétuée. Ann. Soc. Ent. Belg., Tome XXXVI., 1892, pp. 458–461.

Forel, A.

'93 Formicides de l'Antille St. Vincent. Trans. Ent. Soc. London, 1893, Pt. IV., Dec., pp. 333–418.

Headley, F. W.

'01 Problems of Evolution, New York. Thos. Y. Crowell & Co., 1901.

Marchal, Paul.

'96 La Réproduction et l'Evolution des Guêpes Sociales. Arch. de Zool. Exper. et Gén., 3e Ser., Tome IV., 1896, pp. 1–100, 8 fig.

Mayr, G. L.

'62 Myrmecologische Studien. Abh. k.k. Zool. Bot. Ges. Wien. Jahrg., 1862, pp. 649–776, Taf. XIX.

Mayr, G. L.

'66 Diagnosen neuer und wenig gekannter Formiciden. Abh. k.k. Zool. Bot. Ges. Wien., Bd. XVI., 1866, pp. 885–908, Taf. XX.

Mueller, Fritz.

'81 A Metamorphose de un Insecto Diptero. Arch. do Museo Nac. Rio Janeiro, 1881, IV., pp. 47–85, 4 Tab.

Osten Sacken, C. R.

'95 Contributions to the Study of the Liponeuridæ Loew (Blepharoceridæ Loew *olim*). Berl. Ent. Zeitschr., Bd. XL., 1895, Heft I., pp. 148–169.

Schenck.

'52 Beschreibung nassauischer Ameisen. Jahrb. Ver. f. Naturk. in Nassau. Wiesbaden, 1852, Heft VIII., pp. 1–206.

Walsh, B. D.

'62 On the Genera of Aphidæ found in the United States. Proceed. Ent. Soc. Phila., Vol. 1, No. 9, 1862, pp. 294–311.

Williston, S. W.

'86 Synopsis of the North American Syrphidæ. Bull. U. S. Nat. Mus., No. 31, Washington, 1886, pp. xxx, 1–335, Pl. I.–XII.

Austin, Texas,
December 5, 1902.

FUSION OF BLASTOMERES AND NUCLEAR DIVISION WITHOUT CELL-DIVISION IN SOLUTIONS OF NON-ELECTROLYTES.

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I. Introductory.

Blastomeres derived from the cleavage of a single egg-cell have not infrequently been observed to reunite or flow together under various abnormal conditions, producing an apparent reversal of the normal cleavage-process. Thus Dreisch[1] in 1893 observed a refusion of blastomeres in the 4-cell stage of *Echinus* as a result of exposure to temperatures higher than the normal (26 degrees). In 1894 Graf[2] found that compression of *Arbacia* eggs in early stages (16 to 32 cells) also led to a fusion of adjacent cells and even in several instances to a complete reversion from one cleavage stage to the stage immediately preceding (*e. g.*, from 16 cells to 8 cells). In 1896 J. Loeb[3] observed similar phenomena in *Ctenolabrus* eggs deprived of oxygen ; he ascribed the effect to a liquefaction of the cell-membrane and a consequent flowing together of the protoplasm of adjacent cells. Phenomena of a somewhat different though closely related order have also been observed from time to time. Ziegler,[4] while studying the cleavage of enucleated blastomeres, observed that these cells frequently underwent only partial division, the cleavage-furrow forming and subsequently disappearing ; and similar "abortive attempts at division" have been described by other authors.[5] In such instances the cleavage-process appears at first to proceed normally, but it remains incomplete and the cell resumes its original form when the impulse to division ceases.

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[1] Driesch, *Zeitschrift für wissenschaftliche Zoölogie*, 55, 1893, p. 10.

[2] Graf, *Zoölogischer Anzeiger*, 17, 1894, p. 424.

[3] J. Loeb, *Archiv für die gesammte Physiologie*, 62, 1896, p 249.

[4] Ziegler, *Archiv für Entwicklungsmechanik*, 6, 1898, p. 282.

[5] Cf. Wilson, *Archiv für Entwicklungsmechanik*, 12, 1901, p. 529.

Both of the above classes of phenomena present the following general resemblances: the surface-area of the entire egg first of all undergoes a marked increase; increase of area is accompanied by corresponding alterations in the spherical form of the egg; an increase of surface-extent is thus normal to cleavage and indeed may with justification be regarded as one of its most essential features.[1] The increased surface-area does not in the above instances remain unaltered, or increase still further as normally, but undergoes a secondary and abnormal decrease, seen in the smoothing of the incipient cleavage-furrows or the refusion of the already separate blastomeres. These alterations in surface-area are almost undoubtedly to be ascribed to alterations in the surface-tension of the egg. The primary increase of surface seen in cleavage thus indicates a general lowering of the normal surface-tension; this in the above instances appears to be followed by an increase of surface-tension which leads to the succeeding regressive series of changes.

The problem of cleavage thus resolves itself, partially at least if not entirely, into the problem of the nature of the conditions producing alterations of surface-tension at certain definite regions of the egg-surface. It is believed that the experiments about to be described throw light upon this problem. They have demonstrated that cytoplasmic cleavage is prevented in solutions of non-electrolytes, although nuclear division continues; and also that a strong tendency to fusion makes its appearance in the blastomeres of eggs transferred to such solutions in early cleavage-stages. Since under these conditions the electrolytes normally present in the egg must diffuse outward into the surrounding medium, it is to be inferred that cleavage is closely dependent upon the presence of electrolytes within the egg-protoplasm. The manner in which ions derived from the dissociation of the contained electrolytes may conceivably effect alterations in the surface-tension of the egg will be considered in some detail below.

II. EXPERIMENTAL.

The following experiments were performed during the past summer at Wood's Holl as part of a series on which I was then engaged with the aim of determining the influence of the electric current on cell-division in the eggs of *Asterias* and *Arbacia*. Solutions of low conductivity (non-electrolyte solutions with a trace of sea water and isotonic with the latter) were used to insure the passage of the current *through* the eggs. The results of this investigation are incomplete and their publication is deferred for the present. It was found, however, that the simple action of the solutions upon the eggs presented certain interesting peculiarities which form the subject of the following description.

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[1] Compare Rhumbler: Merkel und Bonnet's Ergebnisse, 8, 1898, p. 605 et seq.

The solutions used were molecular solutions (which are approximately isotonic with sea-water) of urea, glycerine, and cane-sugar, especially urea, which has the least injurious action on the eggs.

In these solutions neither starfish nor sea-urchin eggs are capable of cleavage. Eggs transferred to m-urea-solution shortly after fertilization remain living for several hours and nuclear division continues, although more slowly than under normal conditions. No complete cleavages occur, although many eggs, especially those of *Asterias*, assume irregular or amœboid forms. In *Arbacia* a partial constriction may appear at the equator of the egg if the transfer is made shortly before the time of the first cleavage, but in the majority of instances no such signs of incipient cleavage appear.

The following is a record of a typical experiment:

July 12. *Arbacia* eggs, fertilized at 10:27 A. M., were washed with m-urea and transferred to 100 c.c. of the same solution at 11:10 A. M. At 11:35 the control eggs in the sea-water were beginning to segment; in the urea-solutions no signs of segmentation were visible, but a few eggs had become somewhat amœboid in outline. At 11:55 a larger proportion of urea-eggs were amœboid; a certain number were dumb-bell-shaped with a clear area (nucleus) in each enlargement, the division into two halves remaining incomplete. The great majority of eggs showed no such signs of incipient division. At this time the control eggs were in the 2- and 4-cell stages.

Urea-eggs of this series were preserved in picro-acetic and sublimate-acetic fixing fluids,[1] at stages corresponding to the 2-, 4-, 8- and 32-cell of the control. Subsequent examination of stained preparations showed that nuclear division had proceeded in the urea-solutions although cytoplasmic division had been entirely prevented, as above described. In other words, the withdrawal of the electrolytes from the egg in some way prevents the transmission of the division-impulse from the nucleus (which apparently initiates the same) to the body of the cell.

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[1] Boveri's picro-acetic and saturated aqueous mercuric chloride with 2 per cent. glacial acetic acid.

A similar effect has been observed by J. Loeb[1] and Norman[2] to follow the withdrawal of water from the egg by means of sea-water concentrated by the addition of sodium chloride or magnesium chloride; other instances of nuclear division without cell division have been recorded by various authors (Boveri, Chabry, Driesch, Roux).[3] The fact that this phenomenon is seen in solutions of non-electrolytes is of peculiar interest as indicating the importance of the part played in cell-division by the ions present in the cytoplasm. Nuclear division, on the other hand, is apparently independent of the presence of such ions.

Not only is cleavage prevented by transfer to non-electrolyte solutions, but a strong tendency to fusion manifests itself in the blastomeres of eggs transferred during early cleavage stages. This fusion takes place most readily with starfish eggs and in solutions of urea; glycerine- and sugar-solutions are relatively injurious to the eggs and are accordingly less favorable.

The following record will illustrate:

June 27, 1902. Starfish eggs, fertilized at 12:25 P. M., were transferred at 2:15 P. M. while in the 2-, 4-, and 8-cell stages to m-urea-solution. On examination at 3:30 the blastomeres were found in many instances to be completely re-fused, the eggs having apparently reverted to the original unicellular condition. Such eggs form rounded masses of protoplasm each containing several nuclei (which do not fuse) and exhibiting in many cases irregular amœboid projections.

With eggs of more advanced stages (16- and 32-cell) fusion is rarely so complete as in earlier stages. Adjacent blastomeres, however, gradually flow together, and the resulting compound blastomeres, at first hour-glass-shaped, tend to round off, although slowly and as a rule incompletely. Fig. 1 is a representation of an egg with partially fused adjacent blastomeres of the 8-cell stage. This egg presents an unusually regular appearance; typically the fusions follow no definite order and from the 8-cell stage complexes are more frequently obtained that consist of three, four or five more or less rounded and in part compound blastomeres of unequal size, the entire group remaining enclosed within the egg membrane. Contiguity seems to be the chief condition that determines which of the blastomeres undergo fusion; there is no indication that sister blastomeres reunite more readily than cells of different parentage; the fusions take place also after different intervals and with varying degrees of completeness. Thus a strict reversal of cleavage in the sense that the egg reverts to the stage immediately preceding (from 16 to 8 cells, etc.) is of exceptional occurrence. It is of interest to note that fusion is typically preceded by the assumption of a perfectly rounded or spherical form by each cell. The flattening at the surfaces of apposition is thus replaced by a curvature indicative of increased surface-tension (Fig. 2).

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[1] Loeb, J. *Archiv für Entwicklungsmechanik*, Bd. 2, 1896, p. 298.

[2] Norman, *ibid.*, Bd. 3, 1897, p. 106.

[3] For a summary of the observations of these authors cf. Korschelt and Heider, Vergleichende Entwicklungsgeschichte, allgemeiner Theil, erste Lieferung, Jena, 1902, p. 215.

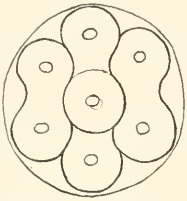

FIG. 1. Example of regular fusion of blastomeres of 8-celled stage. Urea solution. (*Asterias*).

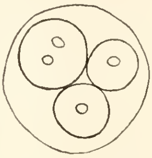

FIG. 2. Rounding of blastomeres in fusion-product of 4-celled stage. Urea-solution (*Asterias*).

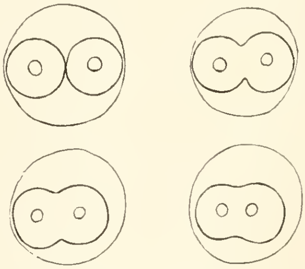

FIG. 3. Different stages of fusion of 2-cell stage in m-glycerine solution (*Asterias*).

In m-glycerine solutions fusions also occur, although in relatively fewer instances. Glycerine is specifically injurious to a far higher degree than urea, and swelling and granular disintegration early appear in many eggs. Different stages of fusion of blastomeres of the 2-cell stage are represented in Fig. 3. Frequently the separate blastomeres merely become rounded without undergoing fusion.

In m-cane-sugar solutions the tendency towards fusion is even less, in consequence apparently of the extreme viscidity and density of the solution and its tendency to withdraw water from the egg. Actual fusion does not occur in this solution; the cells become perfectly spherical, touching only at points of their surfaces — indicating an increase of surface-tension — but without fusing.

In sea-urchin eggs similar phenomena are observed. In general fusion takes place more slowly and less completely in these eggs than in those of *Asterias*. *Arbacia* eggs placed in m-urea solution during the 2- and 4-cell stages exhibit fusion in a large proportion of instances; within thirty minutes after transfer all stages ranging through various dumb-bell-shaped compounds to completely rounded fusion-products are usually seen. Fusions occur also in later stages (16- and 32-cells) in the same manner as in *Asterias*. The blastomeres exhibit a similar tendency to the adoption of a spherical shape before fusion, indicating a preliminary increase of surface-tension.

Fusion occurs also in m-glycerine solutions; separate blastomeres gradually assume the rounded form, and in a few instances they fuse, but fusion is slower and less complete than in urea-solutions. The glycerine acts destructively on these eggs as on those of *Asterias*, leading before long to a swelling and granular disintegration.

III. THEORETICAL.

From the above experiments the conclusion may be drawn that cleavage, as well as the maintenance of the cleaved condition, is dependent upon the presence of electrolytes in the cytoplasm. The activity of these electrolytes must be regarded as due to the ions into which they dissociate. The exact nature of the rôle played by the ions is at present largely a matter of conjecture; but it is reasonable to infer that here, as in other relations, they act chiefly by virtue of the electrical charges which they carry. If this is admitted the following possibilities present themselves for consideration.

If the essential feature of cleavage is an enlargement of the egg-surface due to diminution of surface-tension over certain areas, the possibility at once suggests itself that this alteration of tension may be dependent upon certain known electrical influences,—to be more explicit, upon the appearance of a difference of electrical potential at the surface of contact between the egg and the surrounding medium. That the surface-tension between two adjacent immiscible fluids may be so altered has long been known; the researches of Lippmann and Helmholtz have shown that under these conditions the surface-tension is greatest when the potential-difference of the two adjoining phases is zero, and decreases in definite proportion as the potential-difference increases.[1] The mutual repulsion characteristic of electrical charges of like sign produces at the surface of contact a certain expansive tension which opposes the normal surface-tension and diminishes the latter by that degree. The deformation of the charged mercury-drop, the expansion of the charged soap-bubble, are familiar phenomena dependent upon diminution of surface-tension so occasioned. It is thus possible that the surface-enlargement leading to cleavage may be a special instance of the Lippmann-phenomenon; if this is true we see at once the importance of the electrolytes, since the production of a difference of electrical potential between the two adjoining aqueous media (egg and sea water, separated by a semi-permeable membrane) can be accomplished only by a migration of ions.

These ions must, under the conditions, be situated within the egg; the appearance of a charge at the surface of the latter must therefore signify the gathering of a surplus of ions of one sign in the superficial regions of the egg-protoplasm. This implies the presence of a corresponding surplus of oppositely charged ions in the interior of the egg. Hence the presence of a surplus of ions of either sign in the peripheral region must imply the existence of a difference of electrical potential between the surface and the interior of the egg; and if the conditions of cleavage are as we have supposed, evidence of such potential-difference should be found at the time of cleavage. Is there any evidence of the existence of such difference of potential, and, if so, how is the same to be accounted for?

In answer to the first question it may be said at once that such evidence is by no means lacking, though of an indirect kind and hitherto variously interpreted. It is seen, in my opinion, in the typical appearances presented by the astral radiations and spindle-fibers of dividing cells. The resemblance of the lines of the mitotic figure to the electrical and magnetic lines of force has, needless to say, long been the subject of frequent comment. Now we know that if such an electrical potential-difference between exterior and interior exists, a condition of electrical strain or tension must also exist, the direction of whose lines of traction must correspond with that of the electrical lines of force. It has also been shown that the direction of the fibrils of coagulum in fixed preparations of albumin-solutions subjected during fixation to mechanical strain coincides closely with the direction of this strain (cf. Hardy,[1] Fischer[2]). We may therefore infer from the direction of the fibrils in fixed preparations of dividing cells that a condition of strain or tension exists between interior and periphery of the cell during mitosis, similar to that which would exist in the presence of an electrical potential-difference of the kind imagined above. This agreement confirms the theory that such potential-difference does actually exist. The tendency of the alveoli in echinoderm eggs to dispose themselves along corresponding lines is similarly to be interpreted as an instance of the tendency possessed by polarizable particles in an electric field to arrange themselves in rows along the lines of force.[3]

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[1] Cf. Ostwald, "Lehrbuch der allgemeinen Chemie," 2d Edition, Vol. II., pp. 920–948.

It may fairly be claimed therefore that it is no mere assumption to suppose that during cleavage the surface of the egg is charged differently from the interior. If such a surface-charge is present it will infallibly produce a lowering of the surface-tension with corresponding changes in the form of the egg. Cleavage, on the present theory, is the result of such changes: its conditions will be considered below in further detail.

If we grant then that during mitosis the surface of the egg is charged differently from the interior the question arises: how is this difference of potential established and maintained? In other words, what influence directs ions of one sign toward the periphery, the others toward the interior of the egg? This question appears at present susceptible of only partial reply. It is to be noted however that a marked potential-difference *invariably* makes its appearance (as evidenced by the appearance of astral radiations) whenever the nuclear chromatin passes into the highly condensed and chromatic phase, characterized by a high proportion of nucleic acid, which forms perhaps the most typical and constant of the peculiarities of mitosis. Since, chemically considered, this change denotes an increase in the acidity of the chromatic colloids, and since such acidity implies that the colloidal particles are negatively charged,[1] the inference has seemed reasonable that the inequality of distribution of the ions in the cytoplasm is due to the acquisition of a negative charge of high potential by the colloids composing the nuclear chromatin. I have elsewhere[2] called attention to this possibility and have pointed out that the spiral form of the chromatic filament in the prophase, and also the disposition, mode of division, and movements of the chromosomes, are strongly suggestive of the action of electrostatically charged bodies. If the chromatin carries a negative charge the anions will tend to approach the periphery of the egg as a result of the inductive action of this charge, while the kations will be attracted toward the chromatin. The result will be the establishment of a potential-difference within the cytoplasm of the kind indicated above, with a negative charge at the surface of the egg.

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[1] Hardy, *Journal of Physiology*, 24, 1899, p. 158.

[2] Fischer, Fixierung, Färbung und Bau des Protoplasmas, Jena, 1899.

[3] For experiments bearing directly on this question, see Gallardo, "Interpretacion Dinamica de la division Cellular," Buenos Ayres, 1902. Reviewed by M. Hartog in *Nature*, Vol. 67, 1902, p. 42.

There exists experimental evidence of an apparently conclusive kind that the ions of opposite sign present in a solution may be separated by the inductive action of an electrostatically charged body. The original proof of this proposition appears to be furnished by the well-known and frequently-quoted experiment of Ostwald and Nernst.[3] Recently J. Olsen[4] has described experiments which lead to similar conclusions. This author has found that an electrolyte-solution is affected by an electrostatic charge in such a manner that the liquid nearest the charge assumes a charge of opposite sign to that of the charged body employed, while the remotest portion of the liquid assumes a charge of the same sign as that of the charged body. The liquid, in other words, behaves as if it contained charged bodies free to move (ions) which may be separated by the inductive action of an electrostatic charge, ions of opposite sign moving toward, those of the same sign away from the inducing charge.

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[1] Picton and Linder, *Journal of the Chemical Society*, 1897, LXXI., p. 568. Hardy, *Journal of Physiology*, 24, 1899, p. 288. See also Bredig, "Anorganische Fermente," Leipzig, 1901, p. 15.

[2] *American Journal of Physiology*, 8, 1903, p. 273.

[3] *Zeitschrift für physikalische Chemie*, 3, 1889, p. 120.

[4] *American Journal of Science*, 1902, Vol. XIV., No. 82.

In the cell the charged body on the above theory is represented by the chromatin ; by the inductive action of its negative charge the anions are repelled toward the periphery of the egg while the kations are attracted toward the nucleus. The center of the astral radiations must on this view represent the region of highest positive potential — the region, that is, in which the kations are most densely aggregated. Exactly why there should be *two* such regions, and why these are situated on opposite sides of the nucleus and at some distance from the surface of the latter are questions of considerable difficulty which cannot be considered in detail in the present paper. It is evident that the conditions of electrostatic equilibrium in such a structure as the cell must be of somewhat complex nature. Later I hope to consider these and other questions in fuller detail ; for the present their treatment is deferred.[1]

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[1] I may suggest here briefly that the *form* of the cell must influence the distribution of the surface charges ; and that it is for this reason that the direction of the spindle-axis bears certain well-defined relations to the principal axis of the cell ; these relations have long been known and are formulated in Hertwig's laws of cell-division. If the cell possesses approximately the shape of a prolate ellipsoid — a not infrequent condition — it is to be expected that the repellent action of the internal negative charge will produce a tendency toward aggregation of anions at those regions of the surface-area which are most remote from the central charged body, *i. e.*, which adjoin the long axis of the cell. If the surface negative charge so induced attains sufficient density in these regions there must result a tendency for internally situated kations to be attracted toward the poles of the cell as well as toward the chromatin ; kations will therefore tend to collect in each half of the cell in a certain position of equilibrium at which these opposing tendencies are balanced ; the position of these regions will vary with variations in the electrical condition of the chromatin, but will typically be somewhere between the chromatin and the extremities of the long axis of the cell. The astral centers represent these regions of highest positive potential ; hence they form the centers toward which the electrical lines of force converge. Under the usual conditions they are two in number and adjoin the long axis of the cell. The positions of the two may coincide at their earliest appearance ; hence an originally single aster, situated in close proximity to the nucleus, appears to divide into two which recede toward opposite poles of the cell.

The preponderance of negatively charged ions at the surface of the egg must result in a fall of surface-tension, and it is to this that cleavage on the above theory is supposedly due. That alterations of surface-tension do actually accompany a passage of the chromatin into the chromatic phase, even when normal cleavage fails to occur, seems proved by certain recent observations of E. B. Wilson[1] on artificially fertilized eggs of *Toxopneustes.* It was found that a limited number of eggs after having been exposed to the action of the fertilizing solution (mixture of equal parts sea water and 12 per cent. magnesium chloride) do not segment (as do the majority of eggs thus treated) but undergo the following abnormal changes: "the nuclear area gives rise to a single radiation or monaster which never resolves itself into a bipolar figure. Such eggs never properly segment, but pass through regularly alternating phases of nuclear transformation parallel to those of progressively dividing eggs." That is, first the nucleus enters the resting phase and the astral radiations become greatly reduced; then the nuclear membrane again disappears and the astral radiations regain their original prominence; this is again followed by the reconstruction of the resting nucleus and the reduction of the radiations. The above cycle of changes may occur several times in succession in a single egg. At each disappearance of the nuclear membrane a group of granules appears in the clear center of the aster; these are believed to be chromosomes. These bodies progressively multiply by longitudinal division until finally they may become very numerous. The important fact from our present

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An interesting incidental result of these conditions is a tendency for any minute electrically negative particles casually present in the cytoplasm to be drawn along the lines of force toward the regions of highest positive potential. Here such particles must gather and remain, and in stained preparations they may present the appearance of prominent deeply staining bodies occupying the astral centers. On the above theory the centrosomes originate in some such manner as this. The affinity exhibited by these bodies for basic or nuclear dyes may be regarded as additional evidence of their acidity and electrical negativity. It should be remarked that other authors have regarded the centrosomes as formed by the aggregation of centripetally moving microsomes (cf. Bürger, *Anatomischer Anzeiger*, 1892, p. 222). On the above theory this migration is due to electrical influences. Later, however, I hope to treat these and related questions in a somewhat less summary manner.

[1] Wilson, *loc. cit.*, pp. 546, 547.

standpoint is that "*during the telophase the egg frequently becomes amœboid*, and may even make an abortive attempt to divide. In the later stages in some cases it may actually divide into a number of irregular masses, only one of which contains a nucleus (Fig. 8, *i*), but which here again completely fuse together." These phenomena are almost unquestionably the result of an alteration of surface-tension ; at all events they are precisely what might be expected to occur if such alteration in surface-tension were to take place. It may therefore be regarded as an established fact of observation that during the chromatic nuclear phase certain influences are active which produce alterations in the surface-tension of the egg. That these influences are of an electrical nature may, in view of the above facts and considerations, be regarded as at least highly probable.

The increase in the surface-area of the egg during division may be held to denote a general decrease of surface-tension. But a perfectly uniform decrease of tension over the entire surface could lead to no change of form ; this is evident from the fact that fluid droplets of unequal surface-tension alike tend to assume the spherical form when under the sole influence of such surface-tension, provided this is uniform at all portions of the surface. Changes of form in such droplets result when the surface-tension becomes unequal at different regions of the surface. Thus if the tension is lowered over a small circumscribed area (as appears for instance to occur in the egg during the formation of the polar bodies) the fluid tends to flow outward or be pressed outward at that region — the internal pressure being there insufficiently compensated for the retention of the spherical form — and an amœboid projection is the result. Perfectly definite changes of form, such as occur in cleavage, imply a correspondingly definite localization of the areas of lowered surface-tension. In the case of the egg various indications — such, for example, as the preliminary elongation in the direction of the spindle-axis — point to the conclusion that surface-tension is primarily lowered at the two sides of the egg opposite the astral centers. From the position of the asters during the metaphase and telophase it is to be expected on the present theory that the surface negative charge will be densest near the regions adjoining the long axis of the egg, and that there the surface-tension will accordingly be lowered to the greatest degree. It is also clear that the influence in these regions will increase as the daughter groups of chromosomes approach the poles since the inductive action of the chromatin on the cell-surface must increase as the interval between the two diminishes. The surface-tension at the regions adjoining the astral centers must therefore decrease still further as the daughter groups of chromosomes approach the surface; in other words, the difference between the surface-tension at the polar and at the equatorial regions of the egg (speaking with reference to the spindle axis) progressively increases as the groups of chromosomes diverge. Eventually the egg is surrounded by an equatorial surface-zone possessing a distinctly higher tension — *i. e.*, a stronger tendency to contract — than the polar surface-areas. The effect is naturally the same as would be produced by the presence of a *constricting band* surrounding the equator; a "cleavage-furrow" appears which progressively deepens until complete bipartition is effected.[1]

Cleavage, on this theory, is the result of an inequality of surface-tension between polar and equatorial regions of the dividing cell, due to a greater lowering of surface-tension at the poles than at the equator. This diminution of surface-tension is dependent on the ions present in the protoplasm, hence withdrawal of these, as by the use of non-electrolyte-solutions, prevents cleavage, and, by heightening the normal surface-tension, tends to favor fusion of adjacent blastomeres.

Why similar effects should follow withdrawal of water and compression is not clear. It might be suggested that the effect of compression is an instance of the second of Lippmann's laws cited by Ostwald[2] in his "Lehrbuch": if by mechanical means the surface of the fluid (with tension lowered by the electrical double layer) is increased, the electrical potential-difference of the surface also alters in such a sense as to resist a continued increase of surface, *i. e.*, surface-tension is increased. The increase of surface due to compression of the eggs — supposing the conditions to be of the above kind — must therefore heighten the normal surface-tension ; it will consequently promote fusion. With regard to the effects of withdrawal of water and raising of temperature I have no suggestions to offer at present. Withdrawal of water would result in a decrease in the number of dissociated ions, but it is doubtful if the effect could be attributed to this cause alone.

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[1] So early as 1876 Bütschli (*Abhandl. d. Senkenbergschen Naturf. Ges.*, Bd. 10) referred division to changes of surface-tension due to influences emanating from the astral centers. In his recent paper (*Archiv für Entwicklungsmechanik*, 10, 1900) the equatorial constriction is ascribed to a relative increase in the surface-tension of the equatorial zone of the egg. Conklin (*Journal of the Academy of Natural Sciences of Philadelphia*, Second Series, Vol. XII., Part I., pp. 95 et seq.) is in essential agreement with Bütschli so far as regards the immediate origin of the cleavage furrow.

[2] Ostwald, *l. c.*, p. 923; see also *ibid.*, p. 929.

IV. Concluding Remarks.

On the view briefly sketched above mitosis is an incidental consequence of the passage of the chromatin into the strongly acid and chromatic phase ; this change involves the acquisition by the chromatin of a negative charge of considerable potential, as a result of whose inductive action there ensues a redistribution of the ions in the cytoplasm with the production of certain differences of electrical potential. To these potential-differences are due the appearance of the astral radiations and the diminution of surface-tension that leads to cleavage.

If this theory is well-founded it is evident that the ultimate determining conditions of mitosis must be sought in the conditions that control the chemical changes in the chromatin, especially those affecting the proportion of nucleic acid in this substance. In the mature egg the passage into the chromatic state normally follows the introduction of a spermatozoön ; but the same change may be artificially induced, as shown by J. Loeb and his collaborators, by withdrawal of water, action of certain electrolytes, or even by mechanical agitation. The change is of a metabolic nature and as such is presumably dependent in large part on the action of ferments. We may assume that the liberation of the enzymes concerned, or their activity when liberated, may be dependent on the presence of certain electrolytes. Further speculation on this problem, however, seems premature at present.

Only the most general conditions of mitosis have been considered above. It is evident that, among other conditions, the state of permeability of the egg-membrane to ions, and the metabolic changes in the cytoplasm, resulting as these undoubtedly do in the production of ions, must be considered in a complete theory. It should also be borne in mind that potential-differences in the cytoplasm may conceivably originate otherwise than by induction;[1] that this is actually so is indicated by the phenomena of artificial asters or asters in enucleated eggs (cf. Morgan, Ziegler, Wilson). It seems probable however that in mitosis induction plays the chief part in the production of radiations; the formation of the sperm-aster appears to be another instance of the same phenomenon. In either case astral radiations are to be regarded as the visible expression of local differences of electrical potential within the cytoplasm.

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[1] The localized production (*e. g.*, as an excretory product) of any electrolyte having ions of decidedly different migration-rates would have this effect; it would be especially marked in the case of acids (*e. g.*, lactic acid) on account of the high speed of migration of the hydrogen ion.

THE STRUCTURE AND SIGNIFICANCE OF VESTIGIAL WINGS AMONG INSECTS.

CHARLES T. BRUES.

Although considerable attention has been paid by entomologists to the structure and development of the wings of insects, but few observers have ever given any careful descriptions of insect wings which are in a vestigial condition. The well-marked constancy of the wing structure and its use as an aid to classification has long been recognized, so that one can not fail to suspect *a priori* that there should be a considerable range of variation in the structure of vestigial[1] wings. That the wings are exceedingly important organs has never been questioned, indeed, it is a generally accepted view that they are one of the most important factors in making the Insecta such a dominant group, and the causes which induce their atrophy must undoubtedly be deep-seated. Moreover, from the extent of degeneration it should be possible to obtain some knowledge as to the length of time during which such causes may have been acting. How far such a method may be relied upon, it is my present desire to show.

In a recent paper upon the reduction of wings among the Diptera Bezzi ('00) has shown that there are two categories into which we may divide such Diptera accordingly to the apparent cause of the wingless condition, viz.: First, cases brought about by external parasitism. Second, those induced by a secluded environment: It is possible to include almost all the general types of wingless insects under these two heads, except in a few cases where other influences seem to be at work.

The examination of a number of myrmecophilous Hymenoptera and Diptera has called my attention to the fact that several rather clearly defined types of vestigial wing structure may be recognized. Extending these observations to include other insects also, it seems possible to distribute all these cases into three categories as follows:

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[1] The term vestigial is here used instead of rudimentary. The latter word has often been employed in this connection by entomologists, but with evident impropriety, as it should apply in its strict sense only to organs still in process of ontogenetic development.

1. Wings having essentially a pupal character, *i. e.*, developing as normal wings up to the pupal stage but failing to expand.
2. Wings essentially normal, except for their smaller size and less complex venation; sometimes even developing a color pattern, or possessing unique and quite distinctive characters.
3. Wings consisting of little more than a hollow bag and giving no clue from their appearance as to the probable wing structure of their ancestors. (Comparable in a way to the halteres of the Diptera.)

Of these three groups, the first would seem to indicate the most recently acquired brachypterous condition, and the third apparently the one just preceding the totally apterous state. The latter should therefore be phylogenetically the oldest.

The Trend of Phylogenetic Development in Subapterous Forms.

In flying insects where the wings are of supreme importance their structure is very constant for each form; but as soon as they become vestigial to such an extent that they are no longer available for their only function, that of flight, they are a useless burden, so that once this stage has been reached natural selection should rapidly remove them entirely. This is no doubt the case, for insects with wings just too short for use in flight are very rarely met with. Of these exceptions two groups can be defined:

1. Forms where the wings have suddenly developed characters which make them of use in some other direction.[1]
2. In groups which seem to be undergoing rapid and remarkable phylogenetic changes, *e. g.*, the Phoridæ among the Diptera.

The great preponderance of the forms which have wings of the third type (vide *supra*) shows that there is a rather sharply defined point where they become so vestigial that they are no longer acted upon by natural selection. This also is seen in widely separated groups, *e. g.*, Coleoptera (*Zopherus*) and Diptera (*Ecitomyia*), where the wing is simply a slender, hollow bag of very simple structure.

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[1] Examples of this can be seen in widely separated groups. A notable case is that of a chalcid fly, *Eupelmus rhizophelus*, considered in the sequel.

The fact that in this extremely reduced state they resemble the halteres of Diptera is interesting as affording evidence from another source that the halteres are the vestiges of the second pair of wings which have acquired the new function of equilibration in the Diptera.

The Causes Inducing the Atrophy of the Wings.

Recently Dewitz ('02) has published the results of some experiments upon certain wasps (*Polistes*), where he succeeded in obtaining artificially, specimens of the *Polistes* with abortive wings. These were obtained by subjecting the young larvæ to a low temperature for a considerable period, by laying the nest upon a cake of ice. After undergoing this treatment while still young the insects developed only the stumps of wings on attaining the adult state. Whether the low temperature in this case acts in a way especially to retard the development of the wings other than by causing a general weakness of the body seems to me very doubtful. The anlagen of the wings are present in the pupa and the last and supreme act of an insect on casting off its pupal skin is properly to expand its wings. If its store of energy has been depleted by untoward conditions during its larval life they are the parts which become abnormal. Thus expansion may take place only imperfectly or not at all if the organism has not vigor enough to expand them. I have known artificial conditions of various sorts such as extreme dryness and hot, damp air in closed jars to cause specimens with deformed wings. Under natural conditions such freaks also occur, although much more rarely. During the past summer I have collected a specimen of *Ammophila urnaria* and also one of *Sphex pennsylvanica*, in which the wings were very small and much deformed. Such abnormalities are, however, fundamentally different from normally reduced wings and their structure is not constant. Moreover, such sudden variations without corresponding changes in habits can never be preserved by natural selection.

Dewitz[1] associates a diminution of the oxidation processes in the body with the wingless condition, which is borne out by the comparative anatomy of the tracheal system, but as to which is cause and which effect it is not so easy to decide. As actively flying insects require more air to aërate their blood, they naturally have more extensive tracheæ and respiratory sacs to supply it, so that it seems only proper to consider that in isolated genera or species among a winged group of insects it is the wings and not the tracheæ hidden inside the body which have first been influenced by external conditions and caused to change their form.

In the case of males of certain species which are winged while the females are wingless the short life of the male and the necessity of his seeking the female are sufficient to account for the female being the sex to lose the wings first in becoming adapted to the environment.[2]

It has long been recognized that insects inhabiting certain regions are more apt to be wingless or subapterous than their relatives living under other surroundings; notably the forms inhabiting oceanic islands, mountain tops and of deserts or arid regions tend to have the wings short or wanting. These seem to be the only anomalous cases, for all others can be traced to parasitism or secluded environment. The well-known explanation of Wallace that the great numbers of insects blown out to sea and thus destroyed has caused forms that do not fly or are wingless to be saved at the expense of the more active forms, is regarded by Dewitz ('02) as having little weight. It is true that actual observations upon this point are not numerous, but the immense numbers of dead insects that are often cast up on beaches after a severe storm show that this must be a very important factor. Needham ('00) has given interesting data upon this subject in a recent paper on the insect drift of Lake Michigan in which he describes the enormous numbers of insects cast up on certain portions of the beach of Lake Michigan.[3]

Isolated mountain peaks present similar conditions, although here the mortality can not be so great. A windstorm in such a locality would more readily remove them from their proper environment than in the lowlands, and consequently from conditions favorable for the growth of their offspring. This would favor wingless forms.

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[1] *Loc. cit.*

[2] See also note under *Dicælus*, page 187.

[3] Leconte has described similar phenomena on Lake Superior beaches.

As wingless insects are rarer in cold than in warm regions, the cold does not seem to be a possible factor.

The wingless condition of desert forms has never, I think, been satisfactorily explained, although it is the expression of a well-marked tendency.[1]

A secluded environment very often induces the atrophy of wings. This is seen most strikingly among myrmecophilous and cave insects. There can be but little doubt that in such cases wings are nearly always an inconvenience or even danger. Thus we find that all blind insects are also wingless, wings being evidently detrimental to safety when unguided by vision.

The influence of external parasitism upon the wings is so well recognized that it need only be recalled in the present connection.

From the fact previously alluded to, that the loss of wings is at first very rapid and then suddenly becomes extremely slow when they have reached a very vestigial condition, it can be readily seen that attempts to ascertain the phylogenetic age of a certain wingless type must be very difficult except in a very few cases. Added to this the great differences in plasticity among the widely separated groups with which we have to deal add further difficulties. Nevertheless, by comparing the various cases considered in the second portion of this paper, it is readily seen that the loss of wings must be very readily brought about, for nearly every apterous or subapterous form has a closely related form living under apparently nearly the same conditions, which is winged. Moreover, the loss of wings is usually accompanied by only slight changes in the external morphology of the insect.[2]

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[1] In this connection it may be mentioned not as a full explanation, but as a fact which may bear upon this question; that in wingless coleoptera such as *Eleodes* and allied forms the tight-fitting and immovable elytra must prevent to a great extent the evaporation of water from the body and thus enable them better to withstand long and severe droughts. The fact that *Eleodes* also occurs in certain moist regions can be understood when we remember that arid regions seem to be the centers of distribution for such forms.

[2] In many apterous coleoptera a correlated change is the shortening of the metathoracic or wing-bearing segment.

Thus the causes must be such as affect the wings alone. It seems therefore that we may be justified in saying that it is the result of certain mechanical influences of the environment affecting the wings alone, which causes their loss. This may be accomplished either by hindering the organism in its movements[1] or by laying it open to removal from its proper environment.[2]

First Category, "Pupal" Wings.

Where the development of the wings proceeds normally up to the formation of the pupa and is then suddenly arrested, the adult wings resemble those of the pupa in general detail and form except that they are more heavily chitinized. They occur in certain flies belonging to the family Phoridæ[3] and indicate the first step toward their total atrophy. The species with this form of wings resemble more closely in other morphological details fully winged forms than do their relatives whose wings fall into the second and third categories.

The cases of wingless grasshoppers afford beautiful examples of this persistence of the wing character of the previous instar. In these Orthoptera the wings of subapterous species cannot be distinguished from those of the earlier instars of fully winged species by structure alone. Were it not for the fact that there is a reversion of the relative positions of the two pairs of wings[4] at the last ecdysis one could not decide from a mere examination of the wings whether a certain form is larval or adult.

Empyris subapterus M. et B. MSS., ♀ (Proctotrupidæ) (Fig. 2).

The wings of this species, like those of many other Proctotrupidæ, are very short, but still retain a nearly normal system of neuration. The wing membrane has been lost, while the venation remains undisturbed. This gives the wings a distinctly pupal character.

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[1] That the wings are an inconvenience to life under certain conditions is evinced by the well-known fact that the fertile queens of ants actually tear off the wings on beginning their underground life.

[2] In the cases of insects inhabiting small oceanic islands, mountain tops and deserts, the possible habitat for a species is usually very limited.

[3] Notably the genera *Psyllomyia* Lw. and *Commoptera* Brues.

[4] In all instars except the last, the posterior wings lie above the anterior ones, which position is reversed in the adult.

Megaspilus sp. indescr. (Proctotrupidæ) (Fig. 3).

In a small subapterous species of *Megaspilus* is seen the very common phenomenon of a micropterous species closely resembling other fully winged forms. Many other cases of this could be cited among this group of Hymenoptera.

Apteropompilus (?) sp. indescr. (Fig. 4).

In a most remarkable nearly wingless species of Pompilidæ which occurs in Texas, the venation is more complex than that of *Empyris*, but much simpler than in other genera of Pompilidæ. In this case the wings are very small, being scarcely visible without a strong lens and the thorax is narrower than usual, due doubtless to the slight devolopment of the wing muscles. In this family no intermediate forms are known between this and the fully winged species, all of which fly with great activity. Running has taken the place of flight, a tendency which is seen in many of these sand-wasps which are fully winged.

SECOND CATEGORY.

The following examples have been chosen to illustrate the condition of wings which are much reduced in size, but still retain very well-defined and distinctive characters.

1. *Mutilla grandiceps* Blake, ♂ (Fig. 1).

This species of *Mutilla* is a rare exception among the males of this genus of velvet ants, for like the females of all species, it is incapable of flight. Among all the three or four hundred species of this cosmopolitan group, the rule of wingless females and perfectly winged males holds with only two or three exceptions. From the figure it can be seen that the wings are well proportioned and still retain the hooks upon the anterior margin of the hind pair. The venation is much confused and not plainly defined. The wings while very small in comparison to the size of the insects (reaching scarcely to the tip of the thorax) recall very strongly the normal wings of certain Proctotrupidæ and Chalcididæ where the venation is often completely lost.

2. *Henicopygus subapterus* Ashm. (Chalcididæ) (Fig. 6).

The wings of this insect are very small and evidently useless for flight, but nevertheless have a very distinct venation properly proportioned to their size and exhibit a sharply defined color pattern of hyaline and fuscous patches. They are normal wings in miniature.

3. *Eupelmus rhizophelus* Ashm. (Chalcididæ) (Fig. 7).

One of the most remarkable modifications which I have observed occurs in this insect. The wings which are much reduced in size are suddenly bent upwards at a right angle near the middle and project like two great spines at the apex of the metathorax. The basal half is testaceous in color and flat, while the raised extremities are black and somewhat curled with acute tips. What their function may be I will not venture to suggest, but their extraordinary form leaves little doubt that they are adapted to some special use.

4. Numerous Diptera, *e. g.* Phoridæ (see Brues, '02) and the genus *Termitoxenia*, Wasmann (Fig. 10) show remarkable modifications of the wings which evidently fulfill secondary functions in these insects.

Third Category.

Although all wings falling into this group are necessarily without salient characters, they show a considerable range of variation, which is not apparently correlated, however, with their systematic relationships.

1. *Pasimachus punctulatus* Hald. (Coleoptera) (Figs. 12, 13, 14).

In this carabid beetle the wings are larger than in *Dicælus* (see below) and their larger size is accompanied by a more elaborate form and much greater range of variation, as can be seen from the accompanying figures. They have apparently not yet reached the final minimum size and simple shape. By mounting the wings of specimens which have been preserved in formalin, in glycerine jelly, the tracheation of the wing can be readily made out. A drawing made from such a preparation is shown in Fig. 14. The form of the single trachea was found to be almost identical in several wings; it is a simple tube and not dendriform except for a few slender branches at the tip and at irregular intervals near the middle of the wing where the diameter of the trachea is greatest. The tænidia are very distinct and the tube is considerably coiled upon itself, being somewhat longer than the wing. The development of the wing nervures has evidently been stopped soon after the trachea began to push out into the wing, and the subsequent changes in the trachea not taking place, it has retained its primitive structure. The persistence of a trachea in this manner with its tænidium in an adult insect wing does not seem to have been previously recorded and is especially interesting in this case as it shows the retention of a distinctly pupal character in the imaginal instar; *i. e.*, of arrested development in the wing and not of perfect development on a smaller scale.

2. *Dicœlus splendidus* (Coleoptera, Carabidæ) (Fig. 16).

In this form the wings are similar in the two sexes. They are short, slightly over two millimeters in length or one-sixth the body length, and broadly attached to the integument of the metathorax at their bases. The basal articulation is broad and not at all flexible, and as the wings themselves are very strongly chitinized, they remain immovably fixed and cover the postero-lateral angles of the metathorax. The membrane forming the dorsal wall of the first abdominal segment is pushed inward to give space for the wing, which is quite thick.

Since the other species of this genus, which are winged, make but little use of their wings, it is natural to expect that here both sexes would have the wings reduced to an equal degree, for we are not dealing with a male which flies in search of the female. This points to the idea that it is the difference in the necessary activities of the two sexes that usually causes the male to retain the power of flight longer than the female, and not an inherent morphological or physiological tendency as has been suggested.

3. *Eleodes* sp. (?) (Coleoptera) (Fig. 17).

One of the common Texan species of this large Tenebrionid genus which was examined has extremely short, often bilobed wings which appear simply as slightly projecting protuberances of the metathoracic wall. *Eleodes tricostata* Say and other species have very similar wings.

4. *Zopherus* sp. (?) (Fig. 11).

In this strange Tenebrionid we do not find, as might be expected from the complete coalescence of the elytra, a complete absence of wings. There are structures several millimeters in length, forming thin and delicate flattened sacs which usually show two sharp transverse creases, and very different from the stumps of thick chitin seen in *Eleodes.*

4. *Ecitomyia Wheeleri* Brues (Fig. 9).

The simple type of a hollow bag is seen here in its plainest form, the wing is slightly curled so as to be convex dorsally and concave below, while the upper side is covered with scattered bristles. Otherwise it is structureless. Another Dipteron, *Eretmoptera* recently described by Kellogg has small wings very similar to those of *Ecitomyia.*

EXPLANATION OF PLATE.

Fig. 1. Wing of *Mutilla grandiceps* Bl. ♂.

Fig. 2. Wing of *Empyris subapterus* (M. et B. MSS.), ♀.

Fig. 3. Wing of *Megaspilus* sp. indescr.

Fig. 4. Wing of *Apteropompilus* (?) sp. indescr.

Fig. 5. Wing of a subapterous Braconid. (Chelonus.)

Fig. 6. Wing of *Henicopygus subapterus*, ♀, Ashm.

Fig. 7. Wing of *Eupelmus rhizophelus*, ♀, Ashm.

Fig. 8. Wing of a micropterous ♂ of *Isogenus nubecula.* (After Sharp.)

Fig. 9. Wing of *Ecitomyia Wheeleri* Brues, ♀.

Fig. 10. Wing of *Termitoxenia Heimi* Wasm. (♀ ?). (After Wasmann.)

Fig. 11. Wing of *Zopherus* sp.?

Fig. 12. Wing of *Pasimachus punctulatus.*

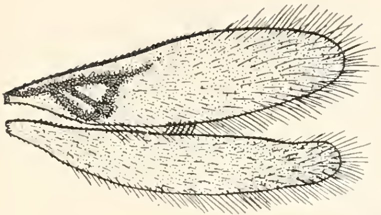
Fig. 13. Wing of *Pasimachus punctulatus*, less enlarged.

Fig. 14. Tracheation of apical part of wing shown in Fig. 12.

Fig. 15. Wing of *Pœcilium affinis*, ♂. (After Sharp.)

Fig. 16. Wing of *Dicælus splendidus.*

Fig. 17. Wing of *Eleodes* sp. (?).



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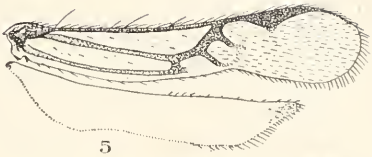

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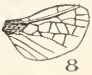

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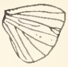

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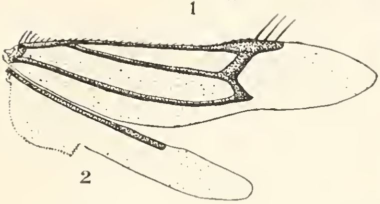

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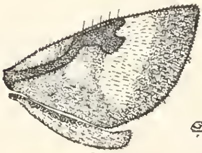

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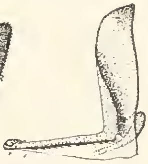

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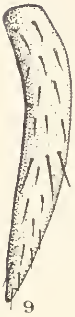

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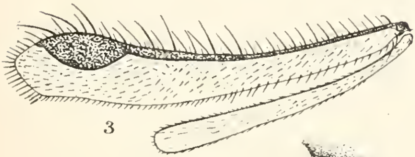

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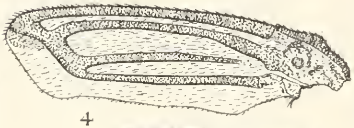

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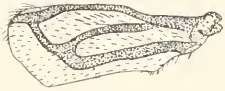

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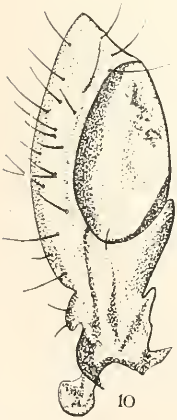

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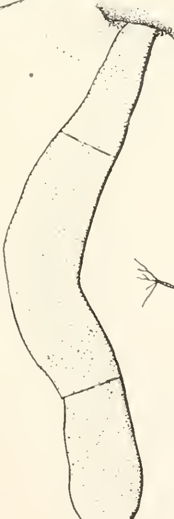

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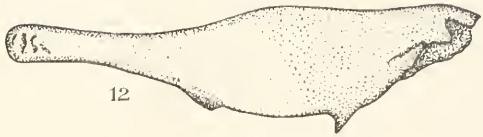

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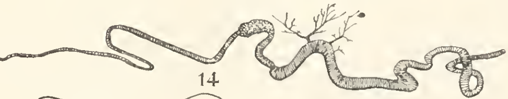

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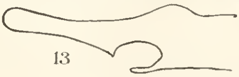

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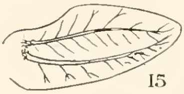

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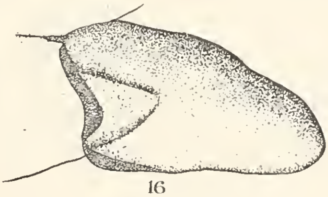

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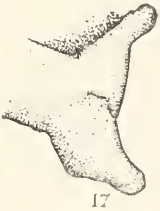

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BIBLIOGRAPHY.

Bezzi, S. C. M.

'00 Sulla Presenza del Genere Chionea Dalman in Italia, e la Riduzione delle Ali nei Ditteri. Rendiconti del R. Ist Lomb. di sc. e lett., Serie II, Vol. XXX., III, 1900.

Brues, C. T.

'02 New and Little-known guests of the Texan Legionary Ants. Amer. Nat., 1902, pp. 365–378.

Casey, T. L.

'90 Coleopterological Notices, II. Annals New York Acad. Sci., V., pp. 307–504, 1890.

Comstock, J. H.

'93 Evolution and Taxonomy, in the Wilder Quarter-Century Book, pp. 37–113, Ithaca, N. Y., 1893.

Comstock, J. H., and Needham, J. G.

'98 The Wings of Insects. A series of eleven papers in the American Naturalist for 1898–1899.

Dewitz, J.

'02 Der Apterismus bei Insekten, seine künstliche Erzeugung und seine physiologische Erklärung. Archiv f. Anat. u. Phys., Phys. abth., pp. 61–67.

Krüger.

'98 Ueber die Entwickelung der Flügel der Insekten mit besonderer Berüchsichtigung der Dechflügel der Kafer. Göttingen, 1898.

Mik, J.

'00 Dipterologische Miscellen. Wien. Ent. Zeit., 1900, pp. 143–145.

Needham, J. G.

'00 Insect Drift on the Shore of Lake Michigan. Memoirs Chicago Ent. Soc., 1900, pp. 19–26.

Speiser, P.

'99 Ueber Reduction der Flügel bei ectoparasitischen Insekten. Insekten-Börse, XVI., pp. 117–122, 1899.

Webster, F. M.

'02 Winds and Storms as Agents in the Diffusion of Insects. Amer. Nat., 1902, pp. 795–801.

Weinland, J.

'90 Beiträge zur Kentniss des Baues des Dipteren Schwingers. Berlin, 1890. Verlag von G. Schade.

Zoölogical Laboratory, Columbia University,
New York, December 19, 1902.

DEATH-FEIGNING IN TERRESTRIAL AMPHIPODS.[1]

S. J. HOLMES.

The instinct of feigning death is extensively distributed throughout the animal kingdom and appears to have arisen sporadically in several groups of animals. It crops out here and there among unrelated forms in such a manner that it is evident that the instinct has arisen independently along many different lines of descent. Various theories of the origin of this instinct have been advanced, but it is by no means evident that the method of its development has been in all cases the same. While at Wood's Hole, Mass., during the past summer my attention was drawn to the death-feigning of the large terrestrial amphipods which occur there in great numbers on the beach, and I was led to study the behavior of these animals with the end of ascertaining, if possible, how their peculiar instinct may have arisen. The family Orchestiidæ, to which the terrestial Amphipoda belong, is partly terrestrial and partly aquatic, and the terrestial forms are, as a rule, confined to within a short distance of the seashore where they live in an atmosphere heavily charged with moisture. The instincts of the terrestrial Orchestiidæ which adapt them to their peculiar habitat must have arisen through certain modifications of the behavior of their aquatic relatives; and as there are several terrestrial and two aquatic species of this family found at Wood's Hole the attempt was made to gain light upon the problem by a comparative study of the behavior of these different forms. I was unable to study the behavior of one of the large sand-fleas, *Talorchestia megalophthalma*, as I failed to obtain any specimens in a living condition. The allied species *T. longicornis* is much more abundant at Wood's Hole and may easily be obtained in any desired quantity. It is the only species observed in which the death-feigning instinct is clearly shown, but the other species, as will appear below, manifest the same fundamental peculiarity, though in an altered form.

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Talorchestia longicornis during the day lies curled up in burrows in the sand situated usually a short distance above high-tide mark where the sand is loose and dry on the surface but moist an inch or more below. The animal remains all day in an apparent stupor much like the sleep of higher animals. The feeding time is at night when *Talorchestias* come out of their holes in swarms and run or hop in a lively manner over the sea-weed near the water's edge. When dug out of its burrows in the daytime *Talorchestia* may lie curled up in its original position, apparently unawakened by the disturbance. Frequently it will make a few hops in the sand and then curl up and lie perfectly quiet. In assuming this condition the body is not only strongly flexed but the legs are drawn up and the antennæ are bent under the thorax, the whole animal assuming as compact a form as possible. While in this attitude *Talorchestia* may be rolled about, picked up and handled, often with considerable roughness, without betraying any signs of animation. It feigns death as perfectly as many of the insects or spiders. After lying in the death-feigning attitude for some time the animal quickly jumps up and scampers away. Waking up, however, is not instantaneous, but is preceded by certain symptoms which may readily be observed if the creature is closely watched. There is first a nervous twitch here and there, then a slight unbending of the antennæ and a straightening of the legs and body — all of which indicate a slight relaxation of the muscular tension under which the animal labors — and finally a sudden spring and attempt to escape. The animal may be induced to feign death by holding it quiet for a moment in the hand or by placing it in the sand. It will feign death many times in immediate succession but the duration of the response becomes on the average less the more often it is caused to repeat the performance. After *Talorchestia* feigns death several times it is more difficult to bring about the response. As it becomes exhausted the death-feigning attitudes become less typical and the body more relaxed and flaccid. The animal is by no means passive while exhibiting this instinct but is in a state of muscular tension; and this condition is, I believe, very common in the death-feigning of other animals. The contact of solid bodies apparently favors the continuation of the feint; at least the duration of the feint is increased when the body of the animal is surrounded with sand or small stones. This fact was determined by placing *Talorchestias* which had feigned death upon a flat surface and counting the number of seconds the feint continued and then comparing the series of observations thus obtained with an equal number of observations made upon specimens partly covered with sand or small stones. Fifty trials were made and the average duration of the feint of the specimens partly surrounded by sand or small stones was found to be much longer than that of the specimens lying on a flat surface. As *Talorchestia* is coming out of its feint a slight pressure or contact causes it to resume feigning.

There is little evidence that the death-feigning of *Talorchestia* is in any way connected with a conscious attempt at deception. Such a performance is utterly beyond what the degree of psychic development which the Amphipoda have probably attained would lead us to expect. The instinctive action of *Talorchestia* which seems most like an intelligent attempt to deceive an enemy is that of crouching upon the approach of a threatening object. *Talorchestia* when running away often crouches to the ground and lies perfectly quiet if a large object draws suddenly near. When things in its environment become quiet again the animal moves on. *Talorchestia* does not feign death upon receiving purely visual impressions; it requires contact of some sort to elicit this form of response. The same fact seems to be quite general in the death-feigning of animals, especially below the vertebrates, and it is a circumstance, I believe, of considerable significance in relation to our views of the genesis of this instinct.

The value of the death-feigning instinct in *Talorchestia* is obvious. When the animal is dug out of the sand its large size would render it an easy prey to an active bird or mammal if it attempted to seek safety in flight; by lying quiet it is, as every one knows who has dug these creatures out, very easily overlooked on account of the resemblance of its color to that of the sand around it. Its death-feigning and its protective coloration both make for concealment and consequently are of service in the life-history of the animal.

The smaller sand-fleas *Orchestia palustris* and *O. agilis*, live in a different situation on the beach from that occupied by the the species of *Talorchestia.* The little *O. agilis* is found in countless numbers beneath the piles of seaweed near the water's edge. This species, as its name implies, is exceedingly active. When disturbed it jumps very rapidly and to such lengths that capturing the creature is an exceedingly difficult undertaking. An enemy which could easily catch the large *Talorchestias* would find the attempt to capture *O. agilis* an unprofitable pursuit. *O. agilis* generally continues hopping until it alights in a place where it can readily get under some object or wedge itself between bodies, so that it secures contact on a considerable surface of its body. Contact seems to exercise a peculiar influence upon this organism, a sort of hypnotic effect apparently, which induces it to flex its body, bend the antennæ downward and lie quiet. The body and antennæ are not so strongly flexed as in the death-feigning of *Talorchestia*, but the same actions are performed though not carried so far. When lying thus *O. agilis* may be disturbed slightly without performing any movement, but an attempt to pick it up or push it about will cause it to quickly "come to" and hop away in the most lively manner. This instinct of *O. agilis* to get into close contact with solid objects is an expression of the strong thigmotactic tendency found among amphipods in general. It is a tendency especially marked in the aquatic representative of the Orchestiidæ, *Allorchestes littoralis.* This species is commonly found under or among stones quite high up on the beach above the range of any of the other aquatic species, thereby showing an approach toward a terrestrial habit. When taken out of the water it is able to perform the exceptional feat of walking upright without falling upon its side, although this is accomplished with some difficulty, and of making leaps into the air like its terrestial relatives. When disturbed it usually moves away by gliding on its side, a movement very common among amphipods which is performed by alternately flexing and extending the abdomen. The efforts are continued until they bring the creature into some niche or crevice where the contact sought for is obtained ; then it curls up and lies quiet. The thigmotactic reactions of amphipods keep these animals among the seaweeds and rocks where they secure protection and obtain food. The behavior of the terrestrial *O. agilis* in relation to solid objects is little modified beyond that of the aquatic species. The thigmotaxis of this form is certainly protective in function, not only by enabling the animal to escape detection by lying quiet, but by leading it into situations such as under stones or into crevices which are inaccessible to its enemies.

The behavior of *Orchestia palustris* shows an interesting connection between that of *Talorchestia* and *O. agilis*. *O. palustris* is considerably larger than *agilis* and is not so active in its movements. It is often found in marshes some distance from the seashore. It usually endeavors to escape by running away and resorts to hopping only under necessity. The tendency to get under or between objects is as strongly developed in this species as in *agilis*, and contact has apparently a stronger quieting effect upon it. When lying quiet *O. palustris* may be poked about more or less without being aroused from its thigmotactic lethargy. Only rarely, however, can it be picked up without its making efforts to escape, although it is much less responsive than *O. agilis*. The conduct of this species is intermediate between the thigmotactic response of *agilis* and the death-feigning of *Talorchestia*. Some specimens might almost be said to possess a death-feigning instinct. The curling up of the body and the bending of the antennæ are not carried so far as in *Talorchestia*, but the same actions are performed which, if carried out in a more decided manner and persisted in longer, would result in what would commonly be called feigning death. The death-feigning instinct of *Talorchestia* is an instinct which, I believe, has its root in the thigmotactic responses common among other amphipods. One may easily conceive that by the selection generation after generation of those individuals of *O. agilis* in which the thigmotaxis is most persistent and in which the body is drawn up in the most compact form during the response a mode of behavior like the death-feigning instinct of *Talorchestia* might readily be produced.

It seems not improbable that an instinct having its phyletic root in a simple thigmotactic response may in course of time come to be comparatively independent of contact stimuli. The persistence of death-feigning in *Talorchestia* depends far less upon contact than the thigmotactic reactions of the aquatic Amphipoda, although, as has been pointed out above, contact still increases the duration of the feint. Contact, finally, may come to be necessary only to set up the instinctive response, having become entirely superfluous for its continuation. And if we conceive the necessary stimulus to be reduced to a single tap or even a jar we can understand how death-feigning reactions such as are found in certain beetles where the response often follows upon the slightest disturbance may have been evolved. Whether they have been so evolved is a question which it would be rash with the evidence in hand to attempt to answer. The singular circumstance that the death-feigning reaction is almost always evoked in response to some form of contact stimulus might be urged in support of such view. The instinct of feigning death has been evolved independently so many times that it is quite possible, if not probable, that it has risen by different methods in different groups of animals. The problem can be solved only by a careful comparative study of death-feigning in several related forms among the various groups of the animal kingdom in which the instinct occurs.

NOTES ON THE REVERSAL OF ASYMMETRY IN THE REGENERATION OF THE CHELÆ IN ALPHEUS HETEROCHELIS.

EDMUND B. WILSON.

We owe to Przibram[1] the interesting discovery that when the larger chela in the genus *Alpheus* (*A. dentipes*, *A. platyrrhynchus*, *A. ruber*) is removed, the chelæ undergo a reversal during the ensuing regeneration, a small chela being regenerated from the stump of the large one, while the former small chela, *which has not been injured*, is directly transformed at the first or second moult into a large one that shows the characteristic structural features of this appendage. A precisely analogous result was obtained in the annelids by Zeleny,[2] who found that after amputation of the functional operculum in *Hydroides* a rudimentary operculum was regenerated in its place, while the rudimentary operculum previously present on the opposite (uninjured) side developed directly into a functional one.

These cases are highly interesting since the reversal of asymmetry involves not merely the enlargement of a smaller structure on the uninjured side, but also profound structural and functional changes due to an injury to another part of the body.

During the summer of 1902 I had an opportunity at Beaufort, N. C.,[3] to repeat Przibram's experiments on *Alpheus heterochelis*, a form in which the differentiation between the two chelæ is extremely marked, and to make some observations on the control of the regenerative process by the nervous system. The anatomy, habits and development of this form have been carefully described by Brooks and Herrick,[4] whose observations give data having an important bearing on the facts to be described.

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[1] "Experimentelle Studien über Regeneration," *Arch. für Entwkm.*, XI., 1901.

[2] "A Case of Compensatory Regeneration in the Regeneration of *Hydroides dianthus*," *Arch. für Entwkm.*, XIII., 4, 1902.

[3] I am indebted to the Hon. G. M. Bowers, United States Commissioner of Fisheries, for the privilege of occupying a table at the Beaufort Laboratory, and to Dr. Caswell Grave, director of the laboratory, for his kind coöperation.

[4] "The Embryology and Metamorphosis of the Macroura," *Mem. Nat. Acad. Sci.*, V., 4.

As shown in Fig. 1, *A*, *B*, the chelæ in this species differ very widely both in size and structure; right-handed and left-handed individuals occur in approximately equal numbers. The large or hammer chela (*B*), which is nearly or quite half the length of the whole animal exclusive of the antenna, is greatly swollen, with a deep transverse groove on either side of the propodus, and a characteristic color-pattern. Both claws are extremely stout, and show very remarkable and characteristic features. On the concave side of the dactylus is a great swollen knob, forming the "hammer" (*h*), which fits into a corresponding deep socket on the outer side of the propodus claw, overhung on the upper side by a prominent setose ridge (*p. s.*). By fully extending the dactylus and then suddenly snapping the claws together the hammer is forced into the socket with such force as to produce a surprisingly loud report, whence the popular name "pistol crab" applied to the animal in some localities (*cf.* Brooks and Herrick).

The large chela has essentially the same structure in both sexes, but the small one shows characteristic sexual differences. In the female (Fig. 2, *D*) it is very straight and slender, and relatively smaller than in the male. In the latter (Fig. 2, *A*) it is not only relatively somewhat larger, but stouter, with relatively shorter claws, the dactylus more strongly curved, and traces of the transverse grooves of the large chela are often present (Fig. 1, *A*). Its most characteristic feature in the male is the presence on both sides of the dactylus of a very marked curved ridge, bearing a series of stiff, short setæ (*d. s.*). A somewhat similar but straighter setose ridge (*p. s.*) is also present on each side of the propodus claw in a position corresponding to that of the setose ridge in the large claw. No trace of the hammer is present in either sex. Ordinarily the small chela alone is used in taking food, the large one being in the main a weapon of offense and defense, as Brooks has graphically described.

As far as the reversal of asymmetry is concerned my observations on *A. heterochelis* entirely confirm those of Przibram on the three species studied by him, but give a slightly different result in cases where both chelæ are amputated. Przibram found (*op. cit.*, p. 331) that in such cases each stump regenerates an appendage of the same type as that which has been removed (*i. e.*, no reversal occurs) but the two are of nearly equal size. The species I have studied agrees in showing no reversal after this operation, but the hammer claw is from the first in most cases very distinctly larger than the other; though as was to be ex-

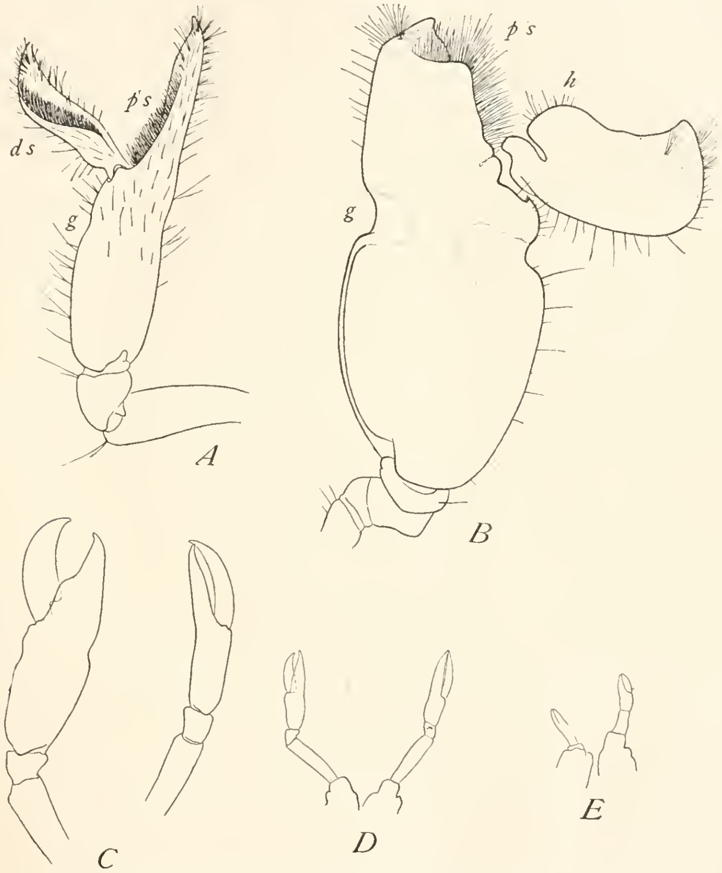

FIG. 1. Normal and regenerating chelæ of *Alpheus heterochelis*, × 4.

A, *B*. Left and right chelæ of male; *d. s.*, setose ridge of dactylus, *p. s.*, setose ridge of propodus; *h*, hammer; *g*, transverse groove, only faintly indicated in the small chela.

C. Chelæ regenerating without reversal, after removal of both appendages from a left-handed specimen; immediately after first moult, twelve days after operation.

D. Chelæ of originally left-handed individual, regenerating very slowly without reversal after removal of both chelæ; eleven days after operation.

E. Regenerating chelæ of originally left-handed individual, three days after removal of both chelæ. Differences of the chelæ already apparent; no reversal (the appendages are viewed from the lower side and hence appear reversed as compared with the other figures).

pected the unequality is very much less marked than in a normal specimen (Fig. 1, *C, D, E*). A comparison of Przibram's Figs. 20–23 (*A. dentipes*), 32–35 (*A. platyrrhynchus*) with mine shows that in point of fact the difference is one of degree only; for in both those species also the figures represent the hammer-claw as slightly larger. The most important addition I am able to make to Przibram's result is the fact that if, after removal of the large chelæ, the nerve of the remaining small chela be cut at the base, *the reversal in some cases at least does not take place, or is incomplete.*

In all, more than a hundred operations were performed, but owing to the large mortality to be expected under the conditions given by the lack of running water in a warm climate not more than half gave definite results, and I shall here report only on such cases.[1]

A first series, including a simple repetition of Przibram's work, gave the following result:

First Series.

A. Of 17 cases in which the large chela was removed, all without exception regenerated in the reversed condition, the remaining small chela being transformed into one of the large type.

B. Of 15 cases in which both chelæ were removed 14 regenerated without reversal — *i. e.*, large and small chelæ reappeared on the same respective sides as before the operation — while a single specimen regenerated reversed.

In three of the cases under *A* the larger chela (originally the smaller) formed on the uninjured side was removed soon after the moult, with the result that a second reversal took place, restoring the original condition. One of these cases is illustrated by Fig. 3, *A–C*. The animal, a female, had originally the large chela on the left side. The first moult occurred nine days after removal of the large chela; Fig. 3, *A*, shows the cast skin of the original small (right) chela, *B*, the same appendage removed three days after the moult, showing the characteristic features of the large chela (transverse groove, shortened and thickened chela, and the "hammer"). The second moult occurred six days later (nine days after the first moult); Fig. 3, *C*, shows the result, the appendage having returned to its original condition, except in size, while a chela of the large type had appeared on the other side.

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[1] The animals were kept singly in bowls, the water being changed once or twice daily, and were abundantly fed on oysters, which were greedily devoured. A complete permanent record of the experiments was kept by preserving every cast skin and the animal that emerged from it, so that there can be no doubt as to the condition of the animal before and after the moult. As a rule the animals were fixed soon after the first moult; but in cases where it occurred soon after the operation they were kept until the second moult. The moults occurred at intervals of 9 to 14 days.

In all cases the hammer-chela on its emergence from the skin of the small chela is less modified than that of a normal animal, always showing characters intermediate between those of the fully developed hammer-chela and the small chela; and the same is true of the regenerating hammer-chela after both chelæ have been removed. In general the longer the period between the operation and the ensuing moult the greater the modification of the hammer-chela. As may be seen from the figures the re-formed or regenerating hammer-chela is less robust than the fully developed one, the claws are longer and less curved, the transverse groove less pronounced and the setose ridge of the propodus (*p. s.*) further removed from the tip of the claw. All these characters become more and more accentuated as the period between operation and moult increases. The shortest observed period in which the regenerating chelæ (after removal of both) showed distinct differences was three days (Fig. 1, *E*); but the difference is here still one of size and general development only. After removal of the large chela only the structural differences may become clearly apparent after five days, as shown in Fig. 2, *G*, *H* (though in this case the remodelled small chela fixed nine days after the moult has probably undergone some further change). In this interesting case the hammer-claw very clearly shows a combination of characteristic characters of the small and large chelæ. It still retains on the whole the general proportions of the small chela of the male, but is more robust, the claws are somewhat shorter, the transverse groove has appeared, and the "hammer" has begun to form. The most interesting point is the retention of the characteristic setose ridge (*d. s.*) of

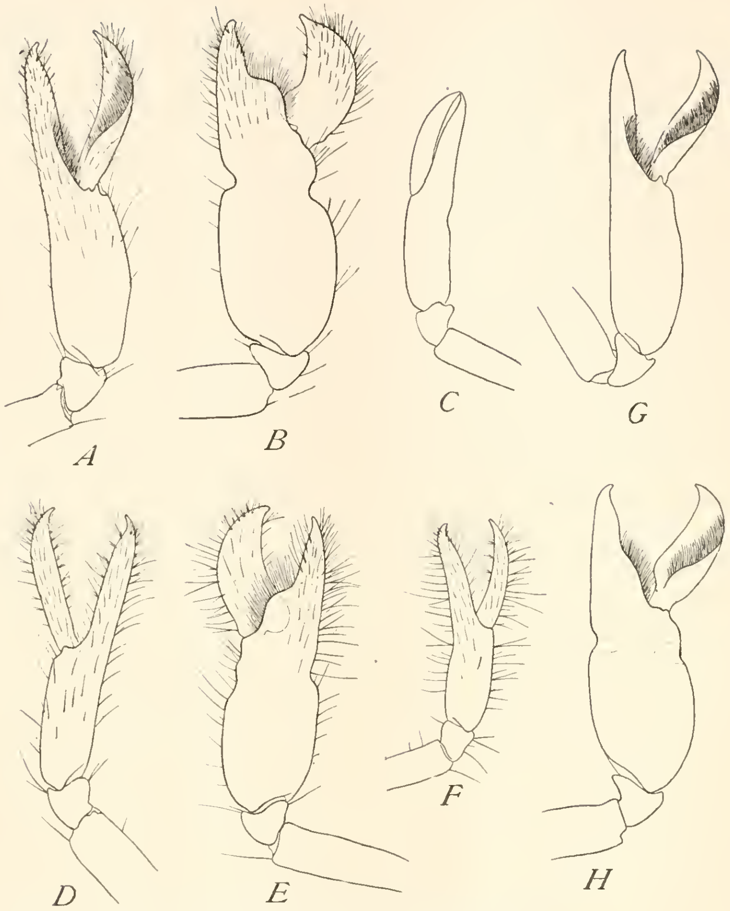

FIG. 2. Typical reversal, after removal of large chela, ×4.

A, *B*, *C*. Reversal in a male, originally left-handed; result of first moult, eleven days after removal of large chela. *A*. Cast skin of original small right chela. *B*. Right chela immediately after moult, showing transformation into hammer-chela well advanced. *C*. Left chela, immediately after moult, regenerated on the stump of the large chela removed.

D, *E*, *F*. Reversal in a female slightly larger than the specimen shown in *A–C*, originally right-handed; result of first moult, sixteen days after removal of large chela. *D*. Cast skin of original small left chela. *E*. Left chela immediately after moult, transformation less advanced than in the male. *F*. Right chela, immediately after moult, regenerated on the stump of the large chela removed.

G, *H*. Initial changes in the typical reversal of an originally left-handed male. *G*. Cast skin of right chela, after first moult, five days after removal of the large left chela. *H*. The same appendage nine days after the moult, showing remodelling in progress, but still retaining the setose ridge on the dactylus.

the dactylus of the male small chela, which is entirely absent in the more modified forms (*e. g.*, in Fig. 2, *B*, *E*). A comparison of the figures will show that in every respect the specimen is intermediate between the unmodified male small chela (Fig. 2, *A*) and the more modified form (Fig. 2, *B*). In specimens of 9 days the setose ridge of the dactylus has wholly disappeared (Fig. 3, *B*) and, except for its somewhat more slender form, is as characteristic as in those of two weeks or more (Fig. 2, *E*).

Second Series.

In a second series the same operations were performed, but in addition the nerve supplying one or both stumps was cut below the base of the appendage. In one set (*C*) the large chela was removed and the nerve of the remaining small chela on the opposite side was cut; in a second set both chelæ were removed and the nerves of both stumps cut. This operation, easily performed by means of a slender sharp-pointed scalpel, results in the first case in a complete paralysis of the remaining appendage. If after a few days control of the appendage was regained, as often happened before the ensuing moult, the operation was as a rule repeated. The mortality in operations of this type is large, and many of the specimens sooner or later cast the remaining appendage; hence only eight successful cases were obtained, and of these only two are beyond question.

C. Of eleven cases in which the large chela was removed and the nerve of the remaining small chela was cut, nine regenerated without reversal, one with reversal, and one with both chelæ of the large type.

D. Of three cases in which both chelæ were removed and the nerves of both stumps cut, all regenerated without reversal. These cases differed from those recorded under *B* only in showing a slight retardation in regeneration.

Of the first nine cases recorded under *C* seven had thrown off the small claw at a varying period before the moult and hence are without value; for such specimens have lost both chelæ. Experiments to test this point show in fact that if the large chela be removed and the smaller one be subsequently removed, after a period sufficiently long to admit of complete reversal under ordinary conditions, the animal regenerates without reversal. For example, from a male left-handed individual the large chela was removed and twelve days later (a period long enough to effect a complete reversal, as shown by other experiments) the remaining small chela was also removed. The animal moulted nine days later (twenty-one days after the first operation) and is *not reversed* showing a typical small chela on the right side and a large one on the left, which however is less modified than usual.

The absence of reversal in the seven cases in which the remaining chela was cast may have been due to the same cause as in the above experiment; but two cases remain, that seem beyond question, though one of these did not moult quite normally and the other not at all. The first case (Fig. 3, *D–F*), a right-handed male, moulted nine days after the operation, but did not succeed in extricating the small chela from the cast skin. The appendage was, however, easily drawn out by hand, in an apparently quite healthy condition, and fixed in formalin. From the right stump of the large chela had been regenerated one of the same type (Fig. 3, *F*), which though still small, shows clearly enough all the characteristic features of the appendage. The appendage drawn out of the cast (Fig. 3, *E*) is on the whole of the small type but has lost the characteristic setose ridge of the dactylus, and shows an indication of the hammer. It is, however, far less modified than the uninjured appendage after the same period of time (Fig. 3, *B*).

The second case, which did not moult, is shown in Fig. 3, *G*, *H*, fourteen days after the operation. The appendage developed on the stump of the large chela, though small, is plainly of the large type. The small (left) chela, owing to the absence of a moult, shows no change.

The foregoing data are too meager to have a very high value, yet they render it probable that the moulding of the small chela into one of the large type, and the production of the small one on the opposite side is controlled by the nervous system — a result in accord with Herbst's remarkable observations on the regeneration of the eye in *Palæmon* and Morgan's on the regeneration of the head in *Allolobophora*. It is possible that the failure of reversal in such operations in *Alpheus* is due to a circu-

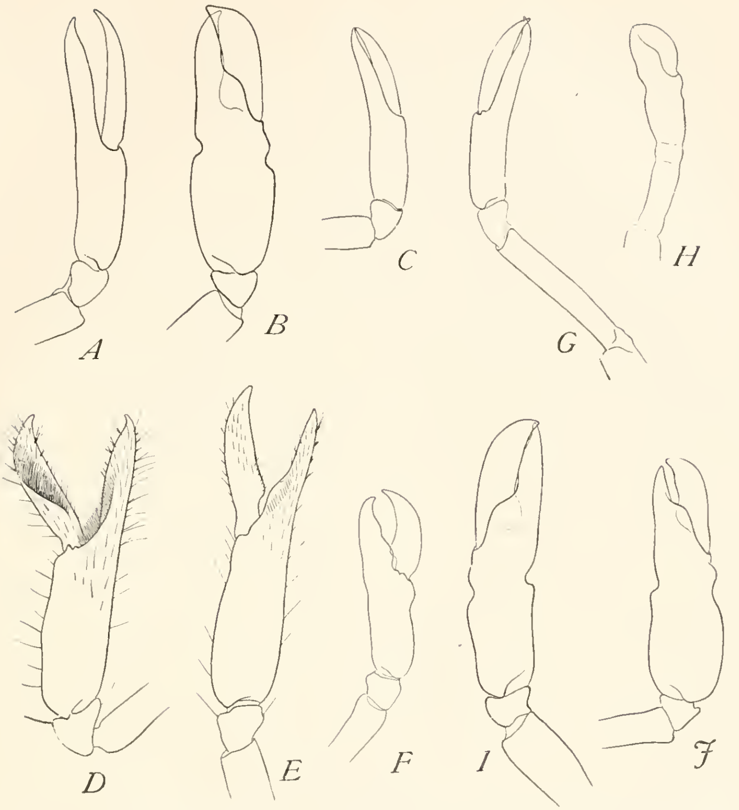

Fig. 3. Normal reversal, and partial inhibition of the reversal after section of nerve of small chela, ×4.

A, *B*, *C*. Double reversal after two successive amputations of large chela. *A*. Cast skin of right chela, female, first moult, nine days after removal of left large chela. *B*. The same appendage amputated three days after moult. *C*. Appendage regenerated on the same stump, immediately after the second moult, six days later; restoration of the original condition. The left chela is again of the large type.

D, *E*, *F*. Partial inhibition of reversal by section of the nerve of the small chela after removal of the large one; originally right-handed male. *D*. Cast skin of small chela at moult, nine days after operation. *E*. The same appendage withdrawn from the cast at the moult. *F*. The right chela, showing distinct features of the large type.

G, *H*. Partial inhibition of reversal after section of nerve. Originally a right-handed female. *G*. The small chela, fourteen days after operation; no moult. *H*. Chela of large type regenerating on stump of large chela removed.

I, *J*. Formation of chelæ transitional to large type on both sides after amputation of large (right) chela and section of nerve of remaining small chela. Originally a right-handed female. Appendages as they appeared after the first moult, fourteen days after operation. Two days before the moult the left chela showed a regain of nervous control.

latory or other non-nervous disturbance. Since, however, the appendage remains in a healthy state and emerges from the moult in a quite normal condition and without sign of atrophy, it seems more probable that the effect is directly due to the lack of nervous control.

The two exceptions recorded above are of considerable interest. In the first case the animal, originally a right-handed female, shows a perfectly typical reversed condition. In the second, the animal, originally a right-handed female, has regenerated both chelæ of the large type, showing the characteristic hammer and other distinctive characters (Fig. 3, *I*, *J*). The left chela (originally the small one) is, however, distinctly less modified than the right, being more slender, with longer and more slender claws, and is in this and other respects clearly intermediate in character between chelæ of the large and small type (Fig. 3, *I*). *Both these specimens, and these alone of those recorded, had regained partial control of the remaining appendage before the moult.* Unfortunately the record does not show precisely how long the control had been regained before the moult. The facts indicate, however, that in the first case the nervous connections had been reëstablished relatively early, so that the typical metamorphosis took place. This case probably gives an interpretation of the second one, which may be explained by supposing that in the early period, before the nervous connections were established, the stump of the large chela (right) had already partially regenerated a chela of the same type (as in the typical case); but that the transformation did not proceed so far as wholly to check the metamorphosis of the small chela (left) upon the reëstablishment of control. Both claws, therefore, reappeared of the large type, that on the right, however, being more modified than the other.

REVERSAL IN RELATION TO THE SECONDARY SEXUAL CHARACTERS.

The experiments bring out some interesting points in relation to the secondary sexual characters (which were not studied by Przibram) and offered data of some importance for the general interpretation of the facts. These concern especially the structure of the small chela, which differs widely in the two sexes.[1]

In the female *Alpheus* this appendage is distinctly less modified than in the male (Fig. 2, *D*), being of slender form, with the claws straighter, weaker and longer. In the male (Fig. 2, *A*), the chela is of more robust form, the dactylus is more curved, and bears on each side a prominent curved ridge armed with a close series of strong setæ, while the fixed claw of the propodite has on each side a similar but straighter and weaker ridge also covered with setæ. Both these ridges are absent in the female. A comparison of Figs. 1, *A*, *B*, 2, *A*, *D*, clearly shows that in some of these respects the small chela of the male is intermediate in type between that of the female and the large chela, which is essentially of the same type in both sexes. This appears in the robuster form of the male chela, the occasional presence of a slight transverse groove on the propodus corresponding to the deep groove on the large chela, the greater thickness and curvature of the dactylus, and the much greater prominence of the seta-bearing ridge on the claw of the propodus, which obviously corresponds to the prominent setose ridge in the corresponding position on the large chela. The female small chela is obviously of more generalized type; and as far as can be judged from the figures of Brooks and Herrick is closely similar to both chelæ of the larval form, on their first appearance.

With these points in mind it is interesting to compare the chelæ of an animal after the first moult subsequent to removal of the large chela. *In both sexes the small chela, regenerated from the stump of the large one, is of the female type* (cf. Figs. 2, *D*, *C*, *F*) *while in both the re-forming large chela is intermediate in form between the fully developed* large chela and the small chela of the male. Furthermore, a comparison of the large chela, in regenerated individuals, shows in general that the longer the period after the operation, the greater its divergence from the small chela. Consideration of the sexual differences brings out the further interesting fact that the transformed small chela of the male for the same period of time is more highly modified than that of the female. This is clearly shown by a comparison of

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[1] *Cf.* Coutière, "Les Alpheidæ," *Ann. Sci. Nat. Zoöl.*, VIII., 9, 1899.

Figs. 2, *B*, 2, *E*. The former shows the transformed small chela of the male, the latter that of the female. The former is in every respect — general proportions, depth of transverse groove, shape and proportion of the claws — more highly modified in the direction of the adult hammer-chela, though the period since the operation was eleven days, while in case of the female it was sixteen.

These facts seem to leave no doubt that the female small chela represents a relatively undeveloped or larval type, that of the male a further development of the same type, accompanied by the appearance of certain specialized secondary sexual features (setose ridge), while the large chela is the extreme of the same line of development. The male small chela is more rapidly transformed into one of the large type, common to both sexes, because it has already advanced further on this line than that of the female.

Comment.

In the above facts we probably find a basis for an interpretation of the reversal of asymmetry during regeneration. The great size of the large chela in *Alpheus*, and its importance as a weapon of offense and defense which Brooks and Herrick pointed out, suggest at once the teleological interpretation that the reversal is a device to secure the least possible delay in the restoration of an important organ by utilizing a structure already present as the foundation of the large chela. That this result is actually effected by the reversal is beyond question; but we need not for this reason assume that the reversal has been specially acquired for this purpose. Structurally the small chela of the female represents a large chela in a state of arrested development, with hardly noticeable modifications of the larval type.[1] That of the male represents a slightly more advanced development along the same line, together with certain special modifications — notably the setose ridge upon the dactylus. In both sexes, accordingly, its transformation into a hammer-chela represents, in its main features, the completion of a process that is inhibited or held in abeyance in the normal condition; though in the male this forward movement is accompanied by a regressive process which causes the disappearance of its specific modifications (setose ridge of the dactylus). To vary the statement, the development of *Alpheus*, at first symmetrical, tends towards a state of equilibrium, characteristic of the species, which is attained through a great inequality in the size of the two chelæ and a series of structural modifications affecting especially the larger one. With the removal of the larger chela the normal equilibrium is reversed and the restorative process proceeds on both sides along the same general lines as in the normal development until a condition of normal equilibrium is restored. A similar interpretation will perhaps apply to Brachyura examined in Przibram's second interesting communication,[1] where it is shown that after the removal of either or both chelæ the regenerated form is always that of the less modified form (which is usually also the smaller). Where only the crushing chela is removed the remaining chela is not transformed into one of the other type; hence no reversal occurs. This case is clearly intermediate between that of *Alpheus*, in which complete reversal occurs, and *Homarus*, in which, as Przibram shows, no reversal occurs, each chela regenerating one of its own type whether one or both is removed. The Brachyura in question (*Carcinus*, *Portunus*, *Eriphia*) exhibit one element of the reversal, namely, the formation of a less modified chela from the stump of the crushing-chela; but fail to complete the metamorphosis of the remaining one. This may be due either to the slowness of the process (which may require a longer time for its completion than that during which the animals were kept under observation) or to a greater rigidity of organization. In *Homarus* the regenerating crushing claw is not in its very early stages recognizable as such, but is of the embryonic type; it assumes many of its characteristic features very early, but for a long period remains of a type intermediate between the two forms of chelæ (*cf.* Przibram's Figs. 13, 14). No reversal occurs. These various cases obviously form a series, at one extreme of which the large chela, after its removal, reappears and remains permanently of the small type, with transformation of the small chela into one of the large type (*Alpheus*); in the Brachyura the first process occurs but not (as far as the observations show) the second; while in *Homarus* the large chela shows transitional characters from a very early period.

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[1] The figures of Brooks and Herrick (*op. cit.*, Plate XX., Figs. 2, 3) of the larval form of this species are not sufficiently detailed to prove this completely, but show both chelæ of equal size and, as far as can be judged, nearly similar in form to the adult female small chela, or the regenerating small chela of both sexes.

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[1] "Experimentelle Studien über Regeneration," II.; *Arch. Entwkm.*, XIII., 1901–02.

In the case of *Alpheus* it is a tempting conclusion that the initial factor (*Auslösung*) that sets in motion the complex process of differentiation of which either side is capable, is primarily only a difference in the amount of material on the two sides.

Mr. C. T. Brues has at my suggestion undertaken a study of the internal changes and has determined the interesting and unexpected fact that the nerves supplying the two chelæ do not differ perceptibly in size; and they appear further not to differ in the number of the component nerve-fibers or the size of the ganglionic centers from which they proceed. As far as the nervous system is concerned, therefore, the adult appears to retain the bilateral symmetry of the larval form, the asymmetry arising through hypertrophy in other tissues, Removal of the large chela obviously reverses the asymmetry in respect to these tissues, and must temporarily at least, lead to a functional nervous difference on the two sides which may be accountable for the release of development in the small chela. This is, however, only a suggestion that must await further test.

The interest of the general interpretation offered above, which is essentially similar to the one suggested by Zeleny in the case of *Hydroides*, seems to me to lie in the explanation that it offers, of a regulative process of undoubtedly high utility to the animal, that is in the main effected by the same factors as those operative in the normal development; and it seems not unlikely that many regulative processes in regeneration may be capable of a like interpretation.

Zoölogical Laboratory, Columbia University,
January 15, 1903.

A PRELIMINARY NOTE ON THE POSITION OF THE PRIMITIVE STREAK, AND ITS RELATION TO THE EMBRYO OF THE CHICK.

FLORENCE PEEBLES.

At the end of the eighth to tenth hour of incubation of the hen's egg, the primitive streak begins to appear as an opaque line extending from the inner edge of the area pellucida to the center of the blastoderm. Between the tenth and eighteenth hours the primitive streak extends in length, until it covers a distance equal to two-thirds of the entire length of the area pellucida. While this increase in length is taking place the primitive groove forms, and at about the twentieth hour the head process is visible as a thickened line extending anteriorly from the primitive streak. I have endeavored to determine, experimentally, the direction of growth, and the exact position of the primitive streak in relation to the later embryo.

Assheton concluded from the results of his experiments that the central point of the area pellucida, before it becomes pear-shaped, represents the anterior end of the primitive streak, and that the latter develops "from the portion of the unincubated blastoderm which lies between the center of the blastoderm and the posterior margin of the area pellucida." Assheton does not mention the number of hours that the eggs were incubated, so that we do not know whether this central point represents the anterior end of the young primitive streak, or the point from which, later, the head process extends. His figures indicate, however, that the central point of the area pellucida represents the anterior end of the completely formed primitive streak of the eighteenth hour, before the head process appears.

In another series of experiments Assheton allowed the embryos to remain in the incubator twenty-four to forty hours after a sable hair had been inserted in the center of the unincubated blastoderm. In the embryos of twenty-four hours' incubation the hair was found at the anterior end of the primitive streak, the medullary groove lying anterior to the hair. In those of forty hours' incubation the point of insertion lay at the level of the most anterior somites. The results of these experiments, as a whole, indicate that when the blastoderm of an unincubated egg is divided into two halves by a plane passed through the center, perpendicular to the long axis of the later embryo, the half anterior to this plane gives rise to the heart, brain, sense-organs, medulla and fore-gut, and all the rest of the embryo posterior to these organs arises through the activity of the primitive streak region.

The conclusions of Assheton are fully substantiated by the results of the experiments which I made in 1898, although at that time I used embryos in which the primitive streak was already present, or completely formed. I found that an injury made at the anterior end of the primitive streak of the eighteenth hour of incubation, appeared in forty-eight hours in the region just back of the heart, between the first pairs of somites.

Kopsch has recently described (1902) a series of experiments which he made in order to determine whether or not the actual cell material of the primitive streak becomes changed into the embryo. This, he concludes, is true, and he is able to show, through these experiments, that the primitive streak represents definite parts of the embryo. The most anterior end becomes the chordal region of the head, from the middle portion the somites and trunk develop, and from the posterior third the parts of the embryo caudal to the twentieth pair of somites are formed. He believes that the young primitive streak represents these regions in greater concentration. Thus, the entire embryo with the exception of the pre-chordal head area arises directly from the cell material of the young primitive streak.

Kopsch recognizes, as all must who attempt experiments on the early embryo of the chick, the great difficulties arising from the variation in degree of development at a given hour, and also the great danger, in the living egg of failure in locating the exact region that is to be injured. These difficulties may account in part for the difference in the results obtained by Kopsch, and by Assheton and myself.

In the spring of 1902 I made a series of experiments in which I used Kopsch's method of injuring certain regions by the introduction of electrodes instead of cauterizing with a hot needle. I was not so successful with this method as I have been with the hot needle. I repeated my own experiments in which the anterior end of the primitive streak of eighteen hours had been killed, and obtained the same result as before; the injury appeared after twenty-four to forty-eight hours in the region of the anterior somites. Kopsch did not injure the anterior end of the primitive streak of an embryo younger than twenty-four hours. He found that an injury made at the anterior end of the primitive streak at this time appears after incubation in the brain, greatly disturbing the development of that region. From this he concludes that the anterior end of the primitive streak represents the chordal region of the head. I have found that an injury made in the area pellucida immediately *in front* of the primitive streak of eighteen hours, appears in this region of the head. It seems reasonable, therefore, to conclude that Kopsch has injured the head process instead of the primitive streak, for by the twenty-fourth hour the former is fully developed.

I can not understand how Kopsch is able to locate the anterior end of the primitive streak in a twenty-four-hour embryo, for eggs incubated in the laboratory at Bryn Mawr, and in my own laboratory in Baltimore, at twenty-four hours show the medullary folds, the first pair of somites, and the notochord; moreover, in the living egg it is almost impossible to determine where the primitive streak ends and the head process begins. After the twentieth hour I have found it impossible, unless development has been delayed, to locate the anterior end of the primitive streak.

I have repeated Kopsch's experiment, and have intentionally injured the distal end of the head process. At the end of twenty-four to forty-eight hours the injured region has been found in the brain, in a position corresponding to that indicated by Kopsch as the result of supposed injury to the anterior end of the primitive streak. If Kopsch had experimented on an embryo of the age indicated by his figures for sixteen and a half hours, I think he would undoubtedly have found that the anterior end of the primitive streak does not, in the later embryo, represent a region beyond the anterior somites, and therefore, does not take part directly in the formation of organs anterior to the heart.

THE WOMAN'S COLLEGE, BALTIMORE, MD.,
January 22, 1903.

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BIOLOGICAL BULLETIN.

NOTES ON MEROGONY AND REGENERATION IN RENILLA.

EDMUND B. WILSON.

H. B. Torrey[1] has published a brief note on regeneration in *Renilla* in which he shows that the young colonies possess a high regenerative capacity and records some valuable preliminary observations, but owing to the difficulty of procuring sufficient material he was unable to carry these observations far enough to reach a decisive result on some of the important questions concerned. During the summer of 1902, after repeated failures in preceding years, I was fortunate enough to secure at Beaufort, N. C.,[2] four lots of fertilized eggs from which hundreds of young colonies were reared (they were kept alive three weeks but produced only the two primary buds) and also a considerable number of older but still young colonies, obtained from the sand. I was thus able considerably to extend Torrey's experiments and also to make observations on the development of egg-fragments that yielded some suggestive results.

(*a*) The Development of Egg Fragments.

All efforts failed to fertilize the eggs artificially, so that only fertilized eggs were available for experiment, but these can easily be cut individually with the scalpel into two or more pieces. As I showed in my paper of 1882[3] the cleavage-nucleus divides several times (from three to five) before cleavage of the cytoplasm occurs, the egg usually segmenting, from two to three hours after it is laid,[4] at once into eight or sixteen blastomeres

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[1] "Some Facts Concerning Regeneration and Regulation in *Renilla*," Biol. Bull., Vol. II., p. 6, 1901.

[2] I am indebted to the Hon. G. M. Bowers, U. S. Commissioner of Fisheries, for the privilege of occupying a table at the Beaufort laboratory, and to Dr. Caswell Grave, director of the laboratory, for his kind coöperation.

[3] "The Development of *Renilla*," *Phil. Trans.*, Vol. III., p. 24.

[4] As I found twenty years ago the eggs are always laid between 5:30 and 6 A. M.

(exceptionally into a larger number), though with many variations. Twenty-four eggs were cut, at intervals of three to ten minutes during the period between discharge and cleavage, into from two to five pieces that were isolated in water containing spermatozoa. Of these fragments at least one piece from each egg developed in all but one case. Of the first nineteen, covering a period of eighty-seven minutes only one piece from each egg developed (eighteen cases). These fragments divided like the whole eggs, and at the same time with the latter (two and one half hours), into approximately eight or sixteen blastomeres. Of the five remaining cases two or three pieces from each egg developed, segmenting in such a way that the total number of blastomeres formed from each egg was at least approximately, and probably accurately, the same as those produced from an entire egg. The number of the blastomeres in the segmenting fragments of the respective eggs (which, owing to lack of time, could not be exactly counted) were approximately 4 + 12, 4 + 12, 4 + 8, 4 + 5 + 16, 4 + 12. These results are what would have been expected. It is evident that the development of only a single piece in the earlier period is due to the fact that the egg still contains only a single nucleus; and only when the nucleus has divided one or more times does the possibility arise of obtaining more than one nucleated piece from a single egg. The facts seem worth recording, as showing that, despite the absence of a fertilization-membrane after fertilization has occurred, non-nucleated pieces of the egg cannot be again fertilized (which agrees with my later experiments on *Cerebratulus*, an account of which is now in press); and also that the period at which cleavage occurs does not depend on the number of nuclei in the piece but on some other progressive change which reaches the critical point at the same time whether the egg be cut to pieces or remain a whole.

Of more general interest is the fact that egg fragments not more than one fourth the bulk of the egg may develop into dwarf larvæ, which, after swimming for the normal period (about forty-eight hours), sink to the bottom, develop tentacles, and *produce the first pair of buds in normal fashion.* Thus arise dwarf colonies of various sizes that are, in every detail, miniatures of the normal colonies of the same age. Fig. 1 shows side by side three colonies eight or nine days old, the largest being he product of a whole egg, the smallest that of a fragment about one fourth the size of a whole egg, and the third that of half an egg. These drawings are from camera sketches that show very nearly the true proportions, though owing to the occasional movements of the animals the details had to be drawn free hand. Allowing for slight differences in the state of expansion it may be seen that the dwarfs are very exactly similar to whole colonies of reduced proportions. Beyond this stage it was not found possible to rear the young colonies, even after three weeks, probably owing to the difficulty of providing the animals with suitable food.

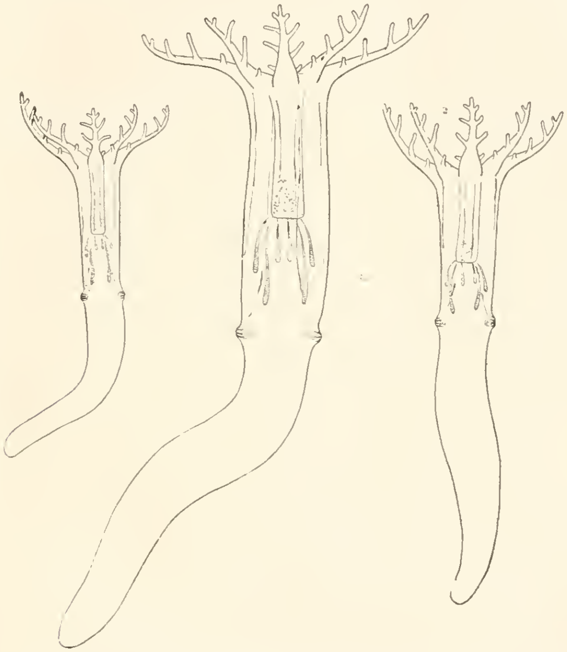

FIG. I. In the middle, primary polyp with first pair of buds, reared from an entire egg, eight days. To the right and left are dwarf colonies nine days old drawn to the same scale, from fragments of fertilized eggs, one of approximately one half, the other of one fourth the bulk of an entire egg.

These results are of some interest as proving that in this case the process of budding does not depend on the attainment of a certain size-limit by the parent stock, but on that of a definite ontogenetic stage, exactly as if the buds were organs of a single individual. It can hardly be doubted that in the ancestral type the buds were set free from the stock, as still occurs in many polyps. Here, however, their individuality has become completely merged in that of the organism as a whole, which develops, as it behaves in the adult condition, essentially as a unit, the bud-formation in the merogonic development being subject to a process of regulation in a manner precisely analogous to the formation of organs in the development of a dwarf pluteus or pilidium from an egg fragment. This conclusion, which is also reached from the facts of regeneration described beyond, is not without a broader interest in its bearing on the possible derivation of metameric animals from linear colonies, or even on the relation of Metazoa to colonial Protozoa.

(*b*) DEVELOPMENT OF FRAGMENTS OF PLANULAS.

I made a few experiments by cutting to pieces the spherical planulas of two to four hours (consisting of 128 cells or more) which are placed on record as suggesting the interest of more extended studies of the same kind. Like fragments of the unsegmented eggs, the planula fragments quickly round out and continue their development for a time apparently unimpaired, and in this way were obtained from a single egg several swimming planulas — in one case nine from a single egg, in one case six, in two cases five, and two or three from the remainder. Though only seven planulas were thus operated the results seem to show that at this period the power of regulation is already somewhat diminished. Of the thirty-one fragments obtained only two developed into normal dwarf colonies. All the others produced abnormal or defective larvæ, the most abnormal ones, as was to be expected, arising from the smaller fragments. Some failed to form stomodæum or septa, others produced stomodæum and the normal number of mesenteries, but no peduncle, several were nearly normally formed but produced no buds, and only a single pair of mesenterial filaments, one produced only a single large median bud, while several of the smaller larvæ showed less than the normal number of mesenteries. Of the nine fragments of a single planula six ultimately died, but the remaining three pieces, including the two largest, developed into larvæ all of which possessed stomodæum and mesenteries. The smallest of these formed no tentacles, peduncle or mesenterial filaments, and only four mesenteries. Both the other two developed six mesenteries and rudimentary tentacles, one pair of short mesenterial filaments; only one of them formed a peduncle. This case indicates that the number of mesenteries is not specified at this period; for although none of the larvæ produced the full number the total number formed from the original egg was sixteen.

(*c*) EXPERIMENTS ON REGENERATION IN THE YOUNG COLONIES.

My principal object in studying the young colonies was to examine the relation between morphallaxis and neomorphosis (to adopt Morgan's terms) for which purpose such an organism as *Renilla* offers obvious advantages, and also to ascertain, if possible, how far the process of bud-formation is capable of regulation. The main results reached by Torrey were: (1) That the polarity of the primary polyps was never reserved, a polyp always regenerating at the anterior end of a piece and a peduncle at the posterior end; (2) that the power of regeneration was confined to the budding zone; (3) that after oblique section, a remoulding of the old parts occurred by a regulation "in a plastic fashion," but he did not succeed in following the later changes long enough to ascertain whether this plastic remoulding was permanent, and involved the establishment of a new axis of symmetry. Like Torrey, I found that when the peduncle was removed, at any level, a new peduncle very quickly reappeared; that removal of the axial polyp by transverse section led to regeneration of that polyp; and that as a rule a severed peduncle did not regenerate a new axial polyp at the anterior end; and that by oblique section through the budding zone two colonies might be produced from one. On some points, however, my results differ from his, while they give a pretty definite answer to the undecided question regarding the phenomena of remoulding.

1. In a single case a reversal of polarity was obtained from a colony having five buds that was cut into three pieces by sections anterior and posterior to the budding zone as shown in Fig. 2, *A*. As a rule after operations of this type, only the middle piece, containing the budding zone, regenerates a perfect colony,

a new peduncle being formed posteriorly and a new axial polyp anteriorly, while neither the severed axial polyp nor the peduncle regenerates, though both may live for a sufficient period of time (in some cases a week or more). In this instance the peduncle, as usual, failed to regenerate, while the middle piece regenerated an axial polyp in front and peduncle behind. The anterior piece (2 *B*) formed an exception to the rule in that it regenerated a large polyp at the posterior end, a form being produced with two similar polyps united at the base and pointing in opposite directions (Fig. 2, *C*). Each possessed a stomodæum, and the mesenteries were continuous from one to the other, bearing six mesenterial filaments. At one side was a rounded elevation that may have been a regenerating peduncle; but the animal died without further change. An interesting feature of this case was the degeneration of the original tentacles, which lost their pinnules and became greatly shortened so as to form rudiments closely similar to those of the regenerated polyp, or those of an early larva. I have observed the same phenomenon in young colonies in which the peduncle, after its removal and regeneration, had been again successively twice removed and regenerated, the whole animal having been in the meantime considerably reduced in size. This shows that regeneration takes place at the cost of material throughout the colony, even involving regressive

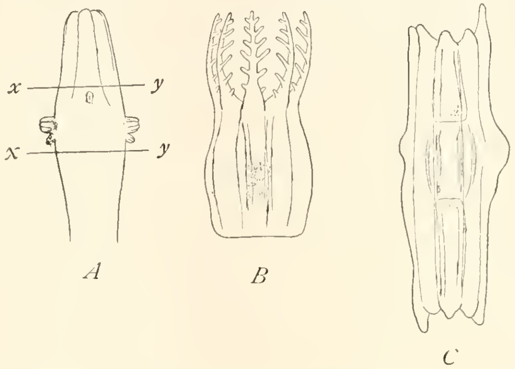

FIG. 2. *A*, outline of young colony with two pairs of buds, in the contracted state, showing planes of section. *B*, the anterior piece (axial polyp), twenty-four hours later; the middle piece already showed a new axial polyp and peduncle well under way. *C*, the anterior piece fifteen days after the operation, with new polyp at base, and the original tentacles reduced.

changes in parts already completely formed. Interesting results can doubtless be obtained by the further study of these changes.

2. The above case not only shows a reversal of polarity but proves that regeneration may take place anterior to the budding zone. A similar result was also given in several cases where the peduncle was removed by section posterior to the budding zone; but this only occurs when the section is not far removed from the budding zone.

In at least one such case it is certain that the section was outside the normal limits of the budding zone, the colony having seven buds on each side. In two days a new axial polyp was well under way at the anterior end of the severed peduncle. In five days this polyp was well developed, with short tentacles and mesenterial filaments, while a pair of symmetrically placed buds had appeared in the same position as in the young colonies (Fig. 3, *D*). A condition was thus attained essentially similar to that of the young colonies developed from the egg (*cf.* Fig. 1), though the peduncle was of course greatly exaggerated in

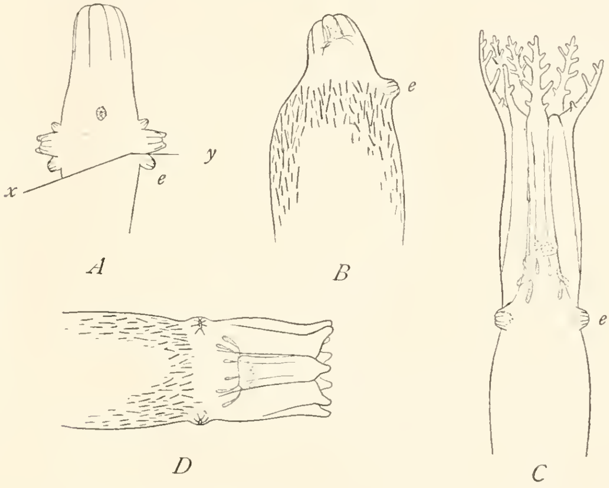

FIG. 3. *A*, outline of young colony with three pairs of buds, showing plane of section. *B*, posterior piece after forty-eight hours, with new axial polyp forming; a new peduncle had formed on the anterior piece. *C*, the same nine days later, with nearly perfect axial polyp and new bud on the left side. *D*, new axial polyp and pair of buds regenerated from the anterior end of a peduncle, cut off behind the budding zone from a young colony with seven pairs of buds; five days after operation.

relative size. This colony died eight days after the operation without further change except further development of the axial polyp.

3. Experiments to test the degree of specification of the persons of the polymorphic colony, and the relation between morphallaxis and neomorphosis, while not absolutely conclusive, give strong ground for the conclusion that, despite a limited power of heteromorphosis and regulation, as shown by the foregoing experiments and those of Torrey, the individual persons are on the whole definitely specified in respect both to the rate of growth and to their axial relations to the colony as a whole. To test this I first tried to see whether the removal of the large buds would result in the more rapid development of the remaining small buds. For this purpose colonies were cut in such a way as to leave only a single small bud attached to the peduncle (Fig. 3 *A*). Since this bud (*c*) is destined to develop into a polyp like the axial one it might have been expected that it would develop at once into a new axial polyp. The fact is quite to the contrary, for the small bud remains stationary while a new axial polyp is produced at the front end of the peduncle (Fig. 3, *B*, *C*). In one case, indeed, the small bud completely disappeared during the process, being apparently absorbed, the whole growth-energy having concentrated in the regenerative process. In the individual figured the small bud remained wholly unchanged while a new axial polyp was regenerated (slightly abnormal in respect to the tentacles), but in the meantime a second bud was formed in the corresponding position on the opposite side of the body; so that the young colony attained the same condition (Fig. 3, *C*) as in Fig. 3, *D*. The comparison of these two cases is interesting, since the same condition was obtained by different methods, both buds being formed anew in the one, while in the other only one bud was produced to form the fellow of the existing one. This indicates that the characteristic first stage in the normal development, with a single pair of buds (Fig. 1) is a definite ontogenetic stage that represents, as it were, a condition of equilibrium that is first restored after the operation before a second step is taken. A definite morphological relation therefore exists between the persons of the

colony that seems exactly comparable to that existing between the different organs of a single individual — essentially the same result as that given by the formation of dwarf colonies from egg fragments.

I endeavored to see whether, by removing all the buds except the exhalent zoöid, this rudimentary person could be made to

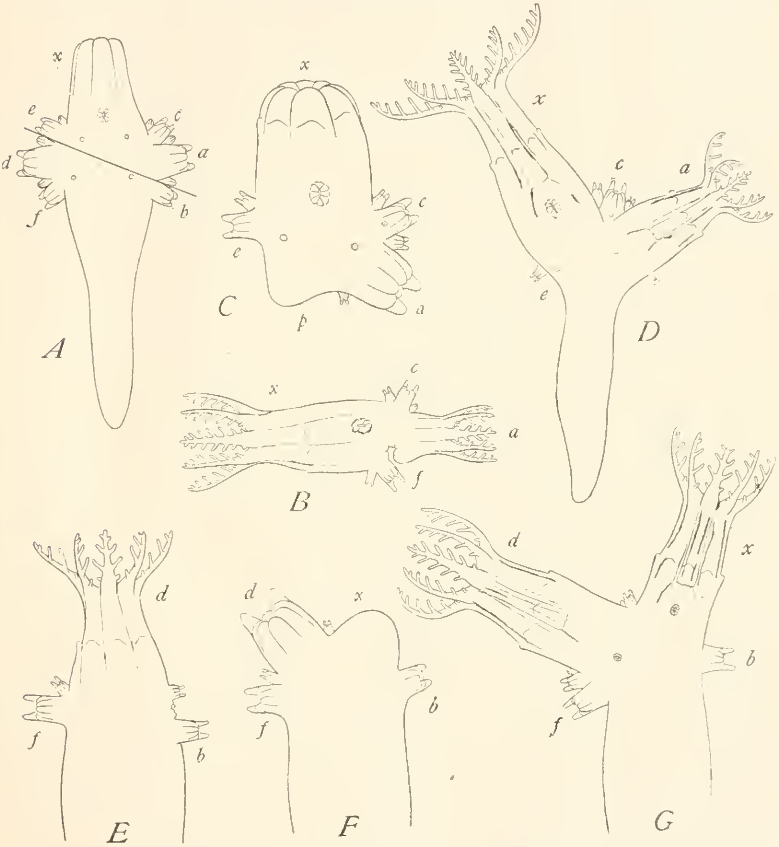

FIG. 4. Regeneration after oblique section. *A*, outline of colony (contracted) with six pairs of buds, showing plane of section. *B*, anterior piece, four hours after operation; after twelve hours the wound had entirely closed. *C*, the same forty-eight hours after operation, showing rudiment of peduncle. *D*, the same five days after operation; return of lateral bud to its original position. *E*, posterior piece, twenty-four hours after operation; lateral bud in the former position of the axial polyp. *F*, the same twenty-four hours later; new axial polyp (*X*) forming. *G*, the same, seven days after operation; new axial polyp well developed, with new exhalent zoöid; return of lateral bud nearly to its original position. During the following ten days the axial polyp became as large as the lateral one, but no other buds were formed.

develop into a fully developed polyp. None of these cases lived more than six days, but in one of them the exhalent zoöid had very considerably enlarged. This may, however, have been a merely passive expansion, and I regret that no decisive result was reached. Repetition of Torrey's experiment of cutting the colony diagonally across at an angle of 45° gave a suggestive but not quite conclusive result. The best case obtained is shown in Fig. 4, the section being in such a plane as to leave one of the primary lateral buds (*a*, *d*) in each piece, the posterior piece having in addition four smaller buds and the anterior one six. Within an hour after the operation, and while the wounds were still widely open, a complete readjustment had occurred in the relative position of buds, and after twelve hours the wounds were entirely healed. The large lateral bud (*d*) of the posterior piece was so displaced as to be directed straight forward, giving exactly the appearance of an axial polyp (Fig. 4, *E*), while by a corresponding process in the anterior piece the lateral bud (*a*) was directed straight backward (4, *B*). In the course of 48 hours however a rapid formation of new tissue took place in both pieces, forming the beginning of a new axial polyp in the posterior piece (4, *F*) and of a new peduncle in the anterior one (4, *C*). At the end of seven days the new axial polyp of the posterior piece (4, *G*, *x*) was fully formed though still not quite as large as the original lateral one, with four pairs of tentacular pinnules, and a median dorsal bud had appeared exactly in the position of the exhalent zoöid. When the colony was fully expanded the new axial polyp was directed almost straight forward while the original lateral bud (*d*) was swung quite over to one side, nearly in its original position, though the colony still showed a very marked asymmetry. At the end of seventeen days the axial polyp was as large as the lateral one, but no other essential change had occurred. The colony was at this time killed for preservation, since I was compelled to leave Beaufort; but the evidence of the specification of the buds obtained from this and the other cases described renders it probable that the original condition would ultimately have been restored and that the primary process of moulding would in the end have been wholly overcome by the regenerative process. In the meantime the anterior piece (4, *D*) had generated a long peduncle, from the anterior end of which the original axial polyp and right lateral one diverged as shown in the figure.

This case shows with great clearness that very soon after the operation the fragment is plastically remoulded, in a manner somewhat similar to that described by Hargitt and Morgan,[1] in the medusa *Gonionemus*, so that without the formation of new tissue the pieces assume a new condition of equilibrium which in the posterior piece brings a lateral polyp into the former position of the axial one. This process takes place so quickly that it seems inadmissible to suppose that it is due to an active process of growth. It gives rather the impression of a mechanical process due to the operation of purely physical factors (tension of the tissue or the like) by which a new condition of equilibrium is restored as nearly as possible like that of an entire colony, and hence representing a case of pure "mechanical regulation" in the sense in which Child has construed the primary process of morphallaxis in planarians.[2] Broadly speaking this process is probably of the same nature as that by which the edges of a cut surface close, though in both pieces the change of form was already complete while the wound was still widely open, as may be seen from Fig. 4, *B*. As such, it cannot be considered as part of the regenerative process in the strict sense,[3] indeed this is proved by the fact that the ensuing regeneration gradually counteracts the effect of the initial remoulding. I can, however, find no ground in Morgan's own discussions for excluding such a mechanical remoulding of the old parts from the conception of morphallaxis in general, and the same ground is taken in the case of *Stenostoma* by Child, who reaches the conclusion that morphallaxis in this form is "essentially a change in form resulting from differences in mechanical tension in the piece as compared with the whole" ('02, p. 414). The facts of initial morphallaxis observed in *Renilla* fall in very well with Morgan's tension-hypothesis, and the remarkable quickness of the process is doubtless due to the extremely plastic character of the tissues in the animal, ordinarily shown in its frequent and very marked changes of form. In planarians the initial remoulding takes place much more slowly and forms an initiative for other regulative changes by which the piece is permanently remodelled into a new form. In *Renilla* such regulative changes are apparently absent, or present in only small degree, and the ensuing process of neomorphosis tends to counteract the initial morphallaxis and restore the original form. Morgan[1] has shown that even two species of the same genus may differ materially in the ratio between remoulding of the old tissues and the formation of new, the latter process being more extensive in *P. lugubris* than in *P. maculata*. *Renilla* appears to offer a case in which both processes occur, but the former produces a result that is only temporary, owing to a high degree of specification in the members of the colony. That this specification is, however, not absolutely fixed is evident from the fact that heteromorphosis may occur, as shown in the formation of a new peduncle from a lateral group of polyps (Torrey), or in the development of a polyp instead of a peduncle from a severed axial polyp; and the same is proved by the establishment of a new colony after removal of the budding zone, as recorded above.

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[1] C. W. Hargitt, "Recent Experiments on Regeneration," *Zoöl. Bull.*, I., 1897. T. H. Morgan, "Regeneration in the Hydromedusa *Gonionemus*," *Am. Nat.*, XXXIII., 1899.

[2] C. M. Child, "Fission and Regulation in *Stenostoma*," *Arch. Entwm.*, XV., 2, 3, 1902.

[3] *Cf.* Morgan, "Regeneration," p. 69.

Zoölogical Laboratory of Columbia University,
January 15, 1903.

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[1] T. H. Morgan, "Growth and Regeneration in *Planaria lugubris*," *Arch. Entwm.*, XIII., 1, 2, 1901.

VARIATION NOTES.[1]

CARL H. EIGENMANN AND CLARENCE KENNEDY.

1. We have received twelve specimens of the cave salamander, *Spelerpes maculicaudus*, from Marble Cave, Mo., and other caves in the neighborhood. With one exception they presented the usual appearance of this species. They were red with dark spots bilaterally unsymmetrically scattered over the back and sides. The spots are subcircular and differ considerably in size. On the sides of the head and body and tail the spots not infrequently become confluent and give rise to short bars usually with their longer axes lengthwise of the specimens. Separating the dotted dorsal surface from the immaculate lower surface, there is an interrupted streak of dark much less intensely pigmented than the spots of the back. In a melanistic specimen from Rockhouse Cave, Mo., this lateral streak has become broad enough to cover the sides with a mottled pattern. The lower surface of the head is always more or less evenly peppered with isolated pigment cells.

The specimen to which attention is called is one exhibiting undoubted melanism. The lower surface of the head is more densely pigmented than in the other specimens. The sides are more uniformly pigmented than in the melanistic individual from Rockhouse. The sides of the head, body, the arms, and anterior surface of the legs are uniformly pigmented, except a few small blotches or spots. The pigmentation is not as intense as in the dorsal spots. The most striking deviation is found on the dorsal surface. The usual spots are present, rather smaller than in the other specimens. The intervening spaces are more densely covered with pigment cells than in the normal specimens and in several places, notably the head, the nape, and one or two places on the back the spots seem to have "*run*," their closely compacted pigment cells having been distributed in a thinner coat over a wider area and formed, with the similarly distributed pigment of other spots, diffuse, evenly pigmented blotches. In life the specimen suggested that the inhibitory force which kept these color cells from spreading, or the positive tropism which kept them together, was dissolved and the cells scattered evenly in a single layer over the surrounding region. The centers of distribution are still distinguishable as darker areas at the margin of or in the blotches. In the nape, for instance, four spots that were in part responsible for the blotch are seen at its four corners. It is very probable that color cells in addition to those originally forming the spots are concerned in forming the blotches.

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[1] Contributions from the Zoölogical Laboratory of Indiana University, No. 52.

2. A specimen of *Pygidium rivulatum*, 195 mm. long, a catfish from Lake Titicaca, has on the left side, in the place of the normal maxillary barbel, one which is dichotomously branched near its base into a dorsal and ventral branch. The dorsal branch is evidently the normal barbel, the ventral being the adventitious one. The ventral branch is but slightly shorter than the dorsal branch which is itself a little shorter than its fellow of the right side.

3. A specimen of *Xiphorhamphus jenynsii*, 155 mm.

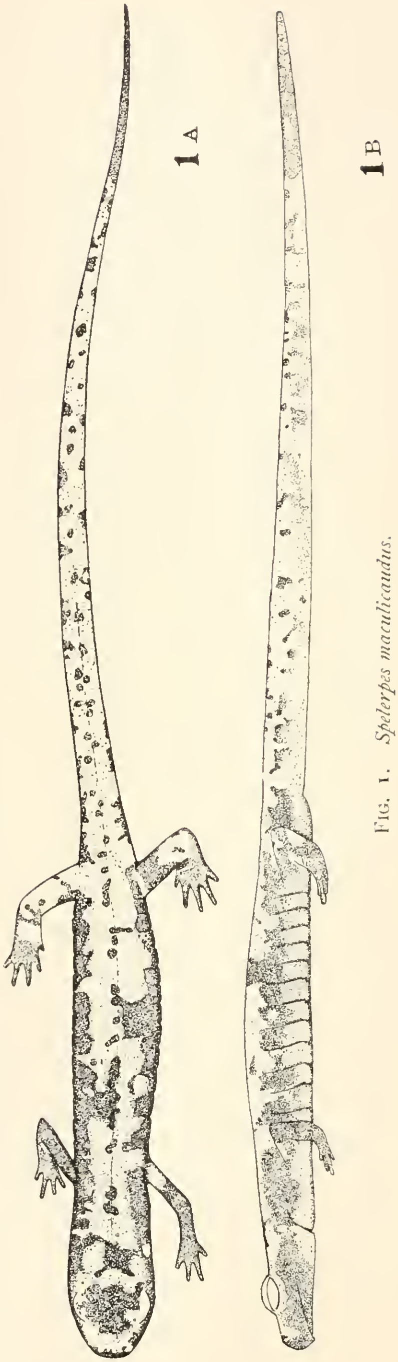

Fig. 1. *Spelerpes maculicaudus.*

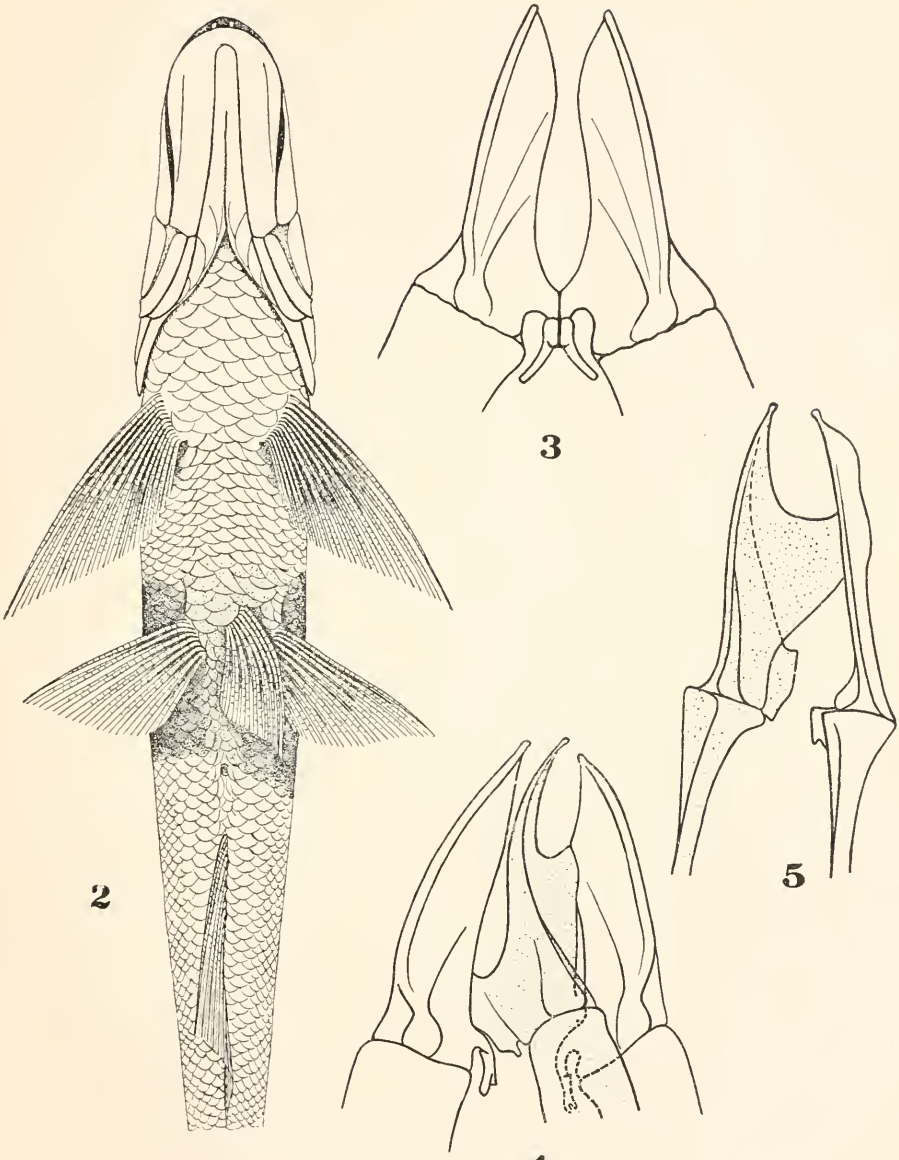

FIG. 2. Ventral surface of a *Xiphorhamphus jenynsii* with an adventitious ventral.

FIG. 3. The innominate bones of a normal *Xiphorhamphus.*

FIG. 4. The innominate bones of the specimen figured in 2.

FIG. 5. Left innominate and adventitious innominate from the left.

long, from the Rio Grande do Sul possesses an adventitious left ventral. The right and left ventrals are normally developed and of equal size. The left ventral is possibly placed a little higher than the right. The adventitious ventral is placed between the two normal ventrals slightly in advance of them and near the left one. It is shorter — 15 mm. as compared with 19 mm. — than the normal ventrals. It possesses one ray less — 8 instead of 9 — than the normal ones and its first ray is bent sickle-fashion.

THE CHROMOSOMES IN HEREDITY.

WALTER S. SUTTON.

In a recent announcement of some results of a critical study of the chromosomes in the various cell-generations of *Brachystola*[1] the author briefly called attention to a possible relation between the phenomena there described and certain conclusions first drawn from observations on plant hydrids by Gregor Mendel in[2] 1865, and recently confirmed by a number of able investigators. Further attention has already been called to the theoretical aspects of the subject in a brief communication by Professor E. B. Wilson.[3] The present paper is devoted to a more detailed discussion of these aspects, the speculative character of which may be justified by the attempt to indicate certain lines of work calculated to test the validity of the conclusions drawn. The general conceptions here advanced were evolved purely from cytological data, before the author had knowledge of the Mendelian principles, and are now presented as the contribution of a cytologist who can make no pretensions to complete familiarity with the results of experimental studies on heredity. As will appear hereafter, they completely satisfy the conditions in typical Mendelian cases, and it seems that many of the known deviations from the Mendelian type may be explained by easily conceivable variations from the normal chromosomic processes.

It has long been admitted that we must look to the organization of the germ-cells for the ultimate determination of hereditary phenomena. Mendel fully appreciated this fact and even instituted special experiments to determine the nature of that organization. From them he drew the brilliant conclusion that, while, in the organism, maternal and paternal potentialities are present in the field of each character, *the germ-cells in respect to each character are pure.* Little was then known of the nature of cell-division, and Mendel attempted no comparisons in that direction; but to those who in recent years have revived and extended his results the probability of a relation between cell-organization and cell-division has repeatedly occurred. Bateson[1] clearly states his impression in this regard in the following words: "It is impossible to be presented with the fact that in Mendelian cases the cross-bred produces on an average *equal* numbers of gametes of each kind, that is to say, a symmetrical result, without suspecting that this fact must correspond with some symmetrical figure of distribution of the gametes in the cell divisions by which they are produced."

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[1] Sutton, Walter S., "On the Morphology of the Chromosome Group in Brachystola magna," BIOL. BULL., IV., 1, 1902.

[2] Mendel, Gregor Johann, "Versuche über Pflanzen-Hybriden," *Verh. naturf. Vers. in Brünn* IV., and in Osterwald's *Klassiker der exakten Wissenschaft.* English translation in *Journ. Roy. Hort. Soc.*, XXVI., 1901. Later reprinted with modifications and corrections in Bateson's "Mendel's Principles of Heredity," Cambridge, 1902, p. 40.

[3] Wilson, E. B., "Mendel's Principles of Heredity and the Maturation of the Germ-Cells," *Science*, XVI., 416.

Nearly a year ago it became apparent to the author that the high degree of organization in the chromosome-group of the germ-cells as shown in *Brachystola* could scarcely be without definite significance in inheritance, for, as shown in the paper[2] already referred to, it had appeared that:

1. The chromosome group of the presynaptic germ-cells is made up of two equivalent chromosome-series, and that strong ground exists for the conclusion that one of these is paternal and the other maternal.

2. The process of synapsis (pseudo-reduction) consists in the union in pairs of the homologous members (*i. e.*, those that correspond in size) of the two series.[3]

3. The first post-synaptic or maturation mitosis is equational and hence results in no chromosomic differentiation.

4. The second post-synaptic division is a reducing division, resulting in the separation of the chromosomes which have conjugated in synapsis, and their relegation to different germ-cells.

5. The chromosomes retain a morphological individuality throughout the various cell-divisions.

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[1] Bateson, W., "Mendel's Principles of Heredity," Cambridge, 1902, p. 30.

[2] Sutton, W. S., *loc. cit.*

[3] The conclusion that synapsis involves a union of paternal and maternal chromosomes in pairs was first reached by Montgomery in 1901.

Montgomery, T. H., Jr., "A Study of the Chromosomes of the Germ-Cells of Metazoa," *Trans. Amer. Phil. Soc.*, XX.

It is well known that in the eggs of many forms the maternal and paternal chromosome groups remain distinctly independent of each other for a considerable number of cleavage-mitoses, and with this fact in mind the author was at first inclined to conclude that in the reducing divisions all the maternal chromosomes must pass to one pole and all the paternal ones to the other, and that the germ-cells are thus divided into two categories which might be described as maternal and paternal respectively. But this conception, which is identical with that recently brought forward by Cannon,[1] was soon seen to be at variance with many well-known facts of breeding; thus:

1. If the germ-cells of hybrids are of pure descent, no amount of cross-breeding could accomplish more than the condition of a first-cross.

2. If any animal or plant has but two categories of germ-cells, there can be only four different combinations in the offspring of a single pair.

3. If either maternal or paternal chromosomes are entirely excluded from every ripe germ-cell, an individual cannot receive chromosomes (qualities) from more than one ancestor in each generation of each of the parental lines of descent, *e. g.*, could not inherit chromosomes (qualities) from both paternal or both maternal grandparents.

Moved by these considerations a more careful study was made of the whole division-process, including the positions of the chromosomes in the nucleus before division, the origin and formation of the spindle, the relative positions of the chromosomes and the diverging centrosomes, and the point of attachment of the spindle fibers to the chromosomes. The results gave no evidence in favor of parental purity of the gametic chromatin as a whole. On the contrary, many points were discovered which strongly indicate[2] that the position of the bivalent chromosomes in the equatorial plate of the reducing division is purely a matter of chance — that is, that any chromosome pair may lie with maternal or paternal chromatid indifferently toward either pole irrespective of the positions of other pairs — and hence that a large number of different combinations of maternal and paternal chromosomes are possible in the mature germ-products of an individual. To illustrate this, we may consider a form having eight chromosomes in the somatic and presynaptic germ-cells and consequently four in the ripe germ-products. The germ-cell series of the species in general may be designated by the letters A, B, C, D, and any cleavage nucleus may be considered as containing chromosomes *A*, *B*, *C*, *D* from the father and *a*, *b*, *c*, *d*, from the mother. Synapsis being the union of homologues would result in the formation of the bivalent chromosomes *Aa*, *Bb*, *Cc*, *Dd*, which would again be resolved into their components by the reducing division. Each of the ripe germ-cells arising from the reduction divisions must receive one member from each of the synaptic pairs, but there are sixteen possible combinations of maternal and paternal chromosomes that will form a complete series, to wit: *a*, *B*, *C*, *D* ; *A*, *b*, *C*, *D* ; *A*, *B*, *c*, *D* ; *A*, *B*, *C*, *d* ; *a*, *b*, *C*, *D* ; *a*, *B*, *c*, *D* ; *a*, *B*, *C*, *d* ; *a*, *b*, *c*, *d* ; and their conjugates *A*, *b*, *c*, *d* ; *a*, *B*, *c*, *d* ; *a*, *b*, *C*, *d* ; *a*, *b*, *c*, *D* ; *A*, *B*, *c*, *d* ; *A*, *b*, *C*, *d* ; *A*, *b*, *c*, *D* ; *A*, *B*, *C*, *D*. Hence instead of two kinds of gametes an organism with four chromosomes in its reduced series may give rise to 16 different kinds ; and the offspring of two unrelated individuals may present 16 × 16 or 256 combinations, instead of the four to which it would be limited by a hypothesis of parental purity of gametes. Few organisms, moreover, have so few as 8 chromosomes, and since each additional pair doubles the number of possible combinations in the germ-products[1] and quadruples that of the zygotes it is plain that in the ordinary form having from 24 to 36 chromosomes, the possibilities are immense. The table below shows the number of possible com-

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[1] Cannon, W. A., "A Cytological Basis for the Mendelian Laws," *Bull. Torrey Botanical Club*, 29, 1902.

[2] Absolute proof is impossible in a pure-bred form on account of the impossibility of distinguishing between maternal and paternal members of any synaptic pair. If, however, such hybrids as those obtained by Moenkhaus (Moenkhaus, W. J., "Early Development in Certain Hybrid Species," Report of Second Meeting of Naturalists at Chicago, *Science*, XIII., 323), with fishes can be reared to sexual maturity absolute proof of this point may be expected. This observer was able in the early cells

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of certain fish hybrids to distinguish the maternal from the paternal chromosomes by differences in form, and if the same can be done in the maturation-divisions the question of the distribution of chromosomes in reduction becomes a very simple matter of observation.

[1] The number of possible combinations in the germ-products of a single individual of any species is represented by the simple formula 2^n in which n represents the number of chromosomes in the reduced series.

binations in forms having from 2 to 36 chromosomes in the presynaptic cells.

Chromosomes.		Combinations in Gametes.	Combinations in Zygotes.
Somatic Series.	Reduced Series.		
2	1	2	4
4	2	4	16
6	3	8	64
8	4	16	256
10	5	32	1,024
12	6	64	4,096
14	7	128	16,384
16	8	256	65,536
18	9	512	262,144
20	10	1,024	1,048,576
22	11	2,048	4,194,304
24	12	4,096	16,777,216
26	13	8,192	67,108,864
28	14	16,384	268,435,456
30	15	32,768	1,073,741,824
32	16	65,536	4,294,967,296
34	17	131,072	17,179,869,184
36	18	262,144	68,710,476,736

Thus if Bardeleben's estimate of sixteen chromosomes for man (the lowest estimate that has been made) be correct, each individual is capable of producing 256 different kinds of germ-products with reference to their chromosome combinations, and the numbers of combinations possible in the offspring of a single pair is 256 × 256 or 65,536; while *Toxopneustes*, with 36 chromosomes, has a possibility of 262,144 and 68,719,476,736 different combinations in the gametes of a single individual and the zygotes of a pair respectively. It is this possibility of so great a number of combinations of maternal and paternal chromosomes in the gametes which serves to bring the chromosome-theory into final relation with the known facts of heredity; for Mendel himself followed out the actual combinations of two and three distinctive characters and found them to be inherited independently of one another and to present a great variety of combinations in the second generation.

The constant size-differences observed in the chromosomes of *Brachystola* early led me to the suspicion, which, however, a study of spermatogenesis alone could not confirm, that the individual chromosomes of the reduced series play different *rôles* in development. The confirmation of this surmise appeared later in the results obtained by Boveri[1] in a study of larvæ actually lacking in certain chromosomes of the normal series, which seem to leave no alternative to the conclusion that the chromosomes differ qualitatively and as individuals represent distinct potentialities. Accepting this conclusion we should be able to find an exact correspondence between the behavior in inheritance of any chromosome and that of the characters associated with it in the organism.

In regard to the characters, Mendel found that, if a hybrid produced by crossing two individuals differing in a particular character be self-fertilized, the offspring, in most cases, conform to a perfectly definite rule as regards the differential character. Representing the character as seen in one of the original parents by the letter A and that of the other by a, then all the offspring arising by self-fertilization of the hybrid are represented from the standpoint of the given character by the formula AA : 2Aa : aa.—that is, one fourth receive only the character of one of the original pure-bred parents, one fourth only that of the other; while one half the number receive the characters of both original parents and hence present the condition of the hybrid from which they sprang.

We have not heretofore possessed graphic formulæ to express the combinations of chromosomes in similar breeding experiments, but it is clear from the data already given that such formulæ may now be constructed. The reduced chromosome series in *Brachystola* is made up of eleven members, no two of which are exactly of the same size. These I distinguished in my previous paper by the letters A, B, C, . . . K. In the unreduced series there are twenty-two elements[2] which can be seen to make up two series like that of the mature germ-cells, and hence may be designated as A, B, C . . . K + A, B, C . . . K. Synapsis results in the union of homologues and the production of a single series of double-elements thus: AA, BB, CC . . . KK, and the reducing division affects the separation of these pairs so that one member of each passes to each of the resulting germ-products.

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[1] Boveri, Th., "Ueber Mehrpolige Mitosen als Mittel zur Analyse des Zellkerns," *Verh. d. Phys.-Med. Ges. zu Würzburg*, N. F., Bd. XXXV., 1902. It appears from a personal letter that Boveri had noted the correspondence between chromosomic behavior as deducible from his experiments and the results on plant hybrids—as indicated also in footnote 1, *l. c.*, p. 81.

[2] Disregarding the accessory chromosome which takes no part in synapsis.

There is reason to believe that the division-products of a given chromosome in *Brachystola* maintain in their respective series the same size relation as did the parent element; and this, taken together with the evidence that the various chromosomes of the series represent distinctive potentialities, make it probable that a given size-relation is characteristic of the physical basis of a definite set of characters. But each chromosome of any reduced series in the species has a homologue in any other series, and from the above consideration it should follow that these homologues cover the same field in development. If this be the case chromosome A from the father and its homologue, chromosome a, from the mother in the presynaptic cells of the offspring may be regarded as the physical bases of the antagonistic unit-characters A and a of father and mother respectively. In synapsis, copulation of the homologues gives rise to the bivalent chromosome Aa, which as is indicated above would, in the reducing division, be separated into the components A and a. These would in all cases pass to different germ-products and hence in a monœcious form we should have four sorts of gametes,

$$\begin{array}{ll} A\,♂ & a\,♂ \\ A\,♀ & a\,♀ \end{array}$$

which would yield four combinations,

$$\begin{array}{rcrl} A\,♂ & + & A\,♀ & = AA \\ A\,♂ & + & a\,♀ & = Aa \\ a\,♂ & + & A\,♀ & = aA \\ a\,♂ & + & a\,♀ & = aa \end{array}$$

Since the second and third of these are alike the result would be expressed by the formula $AA : 2Aa : aa$ which is the same as that given for any character in a Mendelian case. *Thus the phenomena of germ-cell division and of heredity are seen to have the same essential features, viz., purity of units (chromosomes, characters) and the independent transmission of the same;* while as a corollary, it follows in each case that each of the two antagonistic units (chromosomes, characters) is contained by exactly half the gametes produced.

The observations which deal with characters have been made chiefly upon hybrids, while the cytological data are the result of study of a pure-bred form; but the correlation of the two is justified by the observation of Cannon[1] that the maturation mitoses of fertile hybrids are normal. This being the case it is necessary to conclude, as Cannon has already pointed out, that the course of variations in hybrids either is a result of normal maturation processes or is entirely independent of the nature of those divisions. If we conclude from the evidence already given that the double basis of hybrid characters is to be found in the pairs of homologous chromosomes of the presynaptic germ-cells, then we must also conclude that in pure-bred forms likewise, the paired arrangement of the chromosomes indicates a dual basis for each character. In a hypothetical species breeding absolutely true, therefore, all the chromosomes or subdivisions of chromosomes representing any given character would have to be exactly alike, since the combination of any two of them would produce a uniform result. As a matter of fact, however, specific characters are not found to be constant quantities but vary within certain limits; and many of the variations are known to be inheritable. Hence it seems highly probable that homologous chromatin-entities are not usually of strictly uniform constitution, but present minor variations corresponding to the various expressions of the character they represent. In other words, it is probable that specific differences and individual variations are alike traceable to a common source, which is a difference in the constitution of homologous chromatin-entities. Slight differences in homologues would mean corresponding, slight variations in the character concerned — a correspondence which is actually seen in cases of inbreeding, where variation is well known to be minimized and where obviously in the case of many of the chromosome pairs both members must be derived from the same chromosome of a recent common ancestor and hence be practically identical.

In the various forms of parthenogenesis we meet the closest kind of inbreeding and a brief consideration of the variability to be expected in each, from the standpoint of the chromosome theory, may serve as a guide to such research as will test the validity of the latter. The simplest form, of which chemical parthenogenesis in sea-urchins is an examble, is that in which the organism has only a single chromosome series, to be represented by *A*, *B*, *C*, *D* . . . *N*. Thus far no recognized cases of this type have been reared to sexual maturity, but it is to be expected that no reducing division will be found in the maturation of such forms, and that their parthenogenetic offspring will exactly resemble the immediate parent.

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[1] Cannon, W. A., *loc. cit.*

In cases of natural parthenogenesis which are accompanied by the reëntrance of the second polar body and its fusion with the egg-nucleus (or its failure to form) there must be a double chromosome series; but we may distinguish two classes according as the reducing process is accomplished in the first or the second maturation division.[1] If reduction is accomplished in the first division, one half the chromosomes of the oögonia are thrown out and lost in the first polar body. The second division, being equational, would result in a polar body which would be the exact duplicate of the egg-nucleus as far as chromosomes are concerned and which accordingly, by its reëntrance would add nothing new to the egg-series. The series after fusion would, therefore, be represented by the letters $A, B, C, D \ldots N + A, B, C, D \ldots N$. If such a type of parthenogenesis were to follow sexual reproduction, the first generation of offspring might be expected to differ materially from the parent by reason of the casting out, in the first polar body, of chromosomes representing certain dominant characters, and the consequent appearance in the offspring of the corresponding recessives. Subsequent parthenogenetic generations, however, would in each case be endowed with a chromosome series exactly similar to that of the immediate parent and accordingly might be expected to show the same characters.

In case the second division of a parthenogenetic egg were the reducing division, the reëntrance or suppression of the second polar body would accomplish the restoration of the oögonial chromosome-series. In this case the first parthenogenetic generation might be expected to duplicate the characters of the parent (if environmental conditions remained unchanged) and little or no variability would be expected as long as parthenogenesis persisted.

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[1] Either must be regarded as possible in cases where we have no definite knowledge since it is regularly described as the second in the Orthoptera (McClung, Sutton and Copepoda (Rückert, Häcker) while in the Hemiptera-Heteroptera it is believed to be the first (Paulmier, Montgomery).

In relation to these problems there is great need of a simultaneous study of the germ-cell divisions and the variation of periodically parthenogenetic forms.

We have seen reason, in the foregoing considerations, to believe that there is a definite relation between chromosomes and allelomorphs[1] or unit characters but we have not before inquired whether an entire chromosome or only a part of one is to be regarded as the basis of a single allelomorph. The answer must unquestionably be in favor of the latter possibility, for otherwise the number of distinct characters possessed by an individual could not exceed the number of chromosomes in the germ-products; which is undoubtedly contrary to fact. We must, therefore, assume that some chromosomes at least are related to a number of different allelomorphs. If then, the chromosomes permanently retain their individuality, it follows that all the allelomorphs represented by any one chromosome must be inherited together. On the other hand, it is not necessary to assume that all must be apparent in the organism, for here the question of dominance enters and it is not yet known that dominance is a function of an entire chromosome. It is conceivable that the chromosome may be divisible into smaller entities (somewhat as Weismann assumes), which represent the allelomorphs and may be dominant or recessive independently. In this way the same chromosome might at one time represent both dominant and recessive allelomorphs.

Such a conception infinitely increases the number of possible combinations of characters *as actually seen* in the individuals and unfortunately at the same time increases the difficulty of determining what characters are inherited together, since usually recessive chromatin entities (allelomorphs?) constantly associated in the same chromosome with usually dominant ones would evade detection for generations and then becoming dominant might appear as reversions in a very confusing manner.

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[1] Bateson's term.

In their experiments on *Matthiola*, Bateson and Saunders[1] mention two cases of correlated qualities which may be explained by the association of their physical bases in the same chromosome. "In certain combinations there was close correlation between (*a*) green color of seed and hoariness, (*b*) brown color of seed and grabrousness. In other combinations such correlation was entirely wanting." Such results may be due to the association in the same chromosomes of the physical bases of the two characters. When close correlation was observed, both may be supposed to have dominated their homologues; when correlation was wanting, one may have been dominant and the other recessive. In the next paragraph to that quoted is the statement: "The rule that plants with flowers either purple or claret arose from green seeds was universal." Here may be a case of constant dominance of two associated chromatin-entities.

Dominance is not a conception which grows out of purely cytological consideration. Cytology merely shows us the presence in a cell of two chromosomes, either of which is capable of producing some expression of a given character, and it is left to experiment in each case to show what the effect of this combined action will be. The experiment[2] has shown that any one of the three theoretical possibilities may be realized, viz: (1) One or the other may dominate and obscure its homologue. (2) The result may be a compromise in which the effect of each chromosome is to be recognized. (3) The combined action of the two may result in an entirely new cast of character. In cases belonging to the first category, the visible quality (allelomorph, chromatin-entity) was described by Mendel as dominant and the other as recessive, and the experiments of Bateson and Saunders and others, as well as those of Mendel, have shown that in many cases a dominant character tends to remain dominant during successive generations if the environment is not materially changed. Nevertheless, some experiments cited by Bateson[2] go to show that dominance may be variable or defective. Furthermore, it is not only conceivable, but highly probable that in most, if not all cases, there are many different expressions of each character (*i. e.*, many different allelomorphs as suggested by Bateson[3] in regard to human stature), which on various combinations would necessarily exhibit relative dominance. The experiments with peas show an almost constant dominance of certain allelomorphs, such as round over wrinkled in seeds, and of yellow over green in cotyledons; but it is worthy of note that here, as in most Mendelian experiments, only two antagonistic characters have been used. Investigations on varieties, in general similar, but exhibiting different expressions of some particular character, will certainly yield instructive results. Bateson's observations on crosses between single-, rose- and pea-combed fowls, represent a simple form of such a case and may be expected on completion to add much to our knowlege of the nature of dominance.

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[1] Bateson and Saunders, Experimental Studies in the Physiology of Heredity. Reports to the Evolution Committee, I. London, 1902, p. 81, paragraphs 11 and 12.

[2] *Cf.* Bateson and Saunders, *loc. cit.*

In addition to the many examples brought forward by Bateson in support of the Mendelian principle he cites three types of cases which are to be regarded as non-Mendelian. These are:

1. The ordinary blended inheritance of continuous variation.
2. Cases in which the form resulting from the first cross breeds true.
3. The "false hybrids" of Millardet.

1. *Blended Inheritance.* — In treating of this class Bateson clearly states the possibility that the case may be one entirely "apart from those to which Mendel's principles apply," but goes on to show how it may possibly be brought into relation with true Mendelian cases. He says in part: "It must be recognized that in, for example, the stature of a civilized race of man, a typically continuous character, there must certainly be on any hypothesis more than one pair of possible allelomorphs. There may be many such pairs, but we have no certainty that the number of such pairs and consequently of the different kinds of gametes are altogether *unlimited*, even in regard to stature. If there were even so few as, say, four or five pairs of possible allelomorphs, the various homo- and heterozygous combinations might, on seriation, give so near an approach to a continuous curve that the purity of the elements would be unsuspected, and their detection practically impossible." This hypothesis, which presents no difficulties from the point of view of the chromosome theory, is

sufficient in the present state of our knowledge to bring many cases of apparently continuous variation into definite relation with strictly Mendelian cases; but, on the other hand, it seems probable, as already noted (p. 221), that the individual variation in many characters now thought to be strictly Mendelian may prove to be due to the existence in the species of many variations of what may be regarded as the type allelomorphs, accompanying similar variations of the homologous chromatin entities representing those types.

2. *First Crosses that Breed True.* — It is obvious that in the germ-cells of true-breeding hybrids[1] there can be no qualitative reduction. In the normal process synapsis must be accounted for by the assumption of an affinity existing between maternal and paternal homologues, and conversely reduction is the disappearance of that affinity or its neutralization by some greater force. Now in *Hieracium* the characters of the hybrid are frequently intermediate between those of the two parents, showing that both allelomorphs (or chromatin-entities) are at work, but on self fertilization there is no resolution of allelomorphs (reduction division). On the contrary, all the germ-cells are equivalent, as shown by the fact that all combinations produce similar offspring which in turn are similar to the parent. The suggestion made by Bateson in another connection, that "if one allelomorph were alone produced by the male and the other by the female we should have a species consisting *only* of heterozygotes," which would come true as long as bred together, at first sight seems logically applicable to these cases. For such an idea, however, we can find no cytological justification, since if any reduction occurs both chromosomes occur in both male and female germ-cells in equal numbers; and further, the evidence is in favor of a great variety of combinations of maternal and paternal chromosomes in the germ-cells so that the exact chromosome group of a hybrid parent could hardly be duplicated except by fusion of the very pair of cells separated by the reducing division. A more plausible explanation from the cytological standpoint is that the union of the chromosomes in synapsis is so firm that no reduction can take place, *i. e.*, that in each case, a paternal and

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[1] *Cf.* Mendel's experiments on *Hieracium*.

a maternal chromosome fuse permanently to form a new chromosome which subsequently divides only equationally. The result must be germ-cells which are identical with one another and with those of the parents, and hence self-fertilization would produce offspring practically without variation. If this explanation be the correct one the process is distinctly pathological and hence it is not surprising that such cases, as noted by Bateson, should often present "a considerable degree of sterility."

3. *The "False Hybrids" of Millardet.* — Millardet, de Vries and Bateson have all described experiments in which the offspring resulting from a cross between dissimilar individuals showed the character of one parent only, those of the other parent being shown by further experiment to be lost permanently. The obvious cytological explanation of such a phenomenon is hinted at by Bateson in the words "Such phenomena may perhaps be regarded as fulfilling the conception of Strasburger and Boveri, that fertilization may consist of two distinct operations, the stimulus to development and the union of characters in the zygote."[1] Division of the egg without fusion of the pronuclei in a well-known phenomenon having been observed in eggs treated with chloral (Hertwig brothers) or ether (Wilson) and may be supposed to occur under certain unusual conditions in nature. In the experiments mentioned, however, both pronuclei continue to divide separately, while for a cytological explanation of the occurrence of "false hybrids" it is necessary to conceive not only the failure of the nuclei to copulate but the entire disappearance of one of them. Such a case would be comparable to that of chemically induced parthenogenesis or to the fertilization of enucleate egg-fragments, according as the nucleus remaining was maternal or paternal. Speculation in this connection, however, is unprofitable excepting so far as it may serve as a guide to research. A careful study of the fertilization of such cases as Millardet's strawberries, de Vries's *Oenothera* and Bateson's *Matthiola* crosses will no doubt be productive of immediate and positive results.

Mosaics.— A fourth class of non-Mendelian cases, the "mosaics" or "piebalds" constitute a group in relation to which, as I believe, only negative evidence is to be expected from direct cytological study. A good example of the class is the "mosaic" fruit of *Datura* obtained by Bateson and Saunders, which, although in general exhibiting the thornless recessive condition, showed in exceptional cases a thorny patch. Of this case Bateson says: "Unless this is an original sport on the part of the individual, such a phenomenon may be taken as indicating that the germ-cells may also have been mosaic." I must confess my failure to comprehend just what is here meant by mosaic germ-cells. I have attempted to show that in all probability the germ-cells are normally a mosaic of maternal and paternal chromosomes, but very evidently this is not Bateson's meaning.

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[1] Bateson and Saunders, *loc. cit.*, p. 154.

From the standpoint of the chromosome theory I would suggest a possible explanation of the conditions as follows: We have already assumed that the somatic chromosome group, having a similar number of members to that of the cleavage nucleus and derived from it by equation divisions, is made up in the same way of pairs of homologous chromosomes. Every somatic cell, by this conception, must contain a double basis in the field of each character it is capable of expressing. In strictly Mendelian cases one of the homologues is uniformly dominant throughout the parts of the organism in which the character is exhibited. As already noted, however, it is unlikely that all the descendants of a dominant chromatin entity will be dominant. This is shown by the experiment of de Vries with sugar beets, which are normally biennial but always produce a small percentage of annual plants or "runners," which latter are regarded as recessives. The percentage of these runners may be increased by rearing the plants under unfavorable conditions and this is taken as evidence that the recessive allelomorphs may become dominant under such conditions.[1]

If each cell contains maternal and paternal potentialities in regard to each character, and if dominance is not a common function of one of these, there is nothing to show why as a result of some disturbing factor one body of chromatin may not be called into activity in one group of cells and its homologue in another. This would produce just the sort of a mosaic which Bateson and

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[1] *Cf.* Bateson and Saunders, pp. 135, 136.

Saunders found in *Datura* or as Tchermak's pied yellow and green peas obtained by crossing the *Telephone* pea with yellow varieties. Correns describes the condition as *pœcilodynamous* and his conception of the causes of the phenomenon as I understand it is parallel with that which I have outlined above. The logical possibility suggested by Bateson[1] that the recessive islands in such cases as the mosaic pea may be due to recessive allelomorphs in the paired state does not accord with the theory of a chromosomic basis for those allelomorphs, since the chromosome groups, both of cells showing the recessive character and of neighboring cells showing the dominant one, are derived, so far as we know, by longitudinal or equation division from the chromosomes of the same original cleavage nucleus and hence must be alike.

The application of the theory here suggested may be put to test by an experiment in which hybrids of dissimilar true-breeding parentage are crossed and a third generation of "quarter-bloods" produced. Mosaics occurring in such an organism, if this theory be correct, would show one character resembling that of one of the maternal grandparents and one resembling that of one of the original pure-breds of the paternal side. If both characters of the mosaic should be clearly paternal or maternal the theory as outlined is proven inadequate, since one of each pair of chromosomes, and hence the corresponding character-group, is thrown out by the reduction-division in each generation.

In considering the behavior of the two chromosomes forming the basis of any given character, it was noted that in some cases the heterozygote character resulting from the combinations of dissimilar allelomorphs is sometimes totally unlike either of the latter. Thus Mendel found that in crosses between peas respectively 1 and 6 feet in height the offspring ranged from 6 to 7½ feet. In discussing similar cases, Bateson calls attention to the light which would be thrown on the phenomenon if we ventured to assume that the bases of the two allelomorphs concerned are chemical compounds ; and he compares the behavior of the allelomorphs to the reaction of sodium and chlorine in the formation of salt. The results of chemical analysis show that one of the most characteristic features of chromatin is a large percentage content of highly complex and variable chemical compounds, the nucleo-proteids, and therefore if, as assumed in the theory here advanced, the chromosomes are the bases of definite hereditary characters, the suggestion of Bateson becomes more than a merely interesting comparison.

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[1] Bateson and Saunders, p. 156.

We have seen reason in the case of the true-breeding hybrids to suspect that the transmission by the hybrid of heterozygote characters may be due to permanant union of the homologous chromosomes. From this it is but a short step to the conclusion that even if, as is normally the case, the chromosomes do not fuse permanently, the very fact of their association in the same liquid medium may allow a possibility of a certain degree of chemical interaction. This must normally be slight, since its effects do not appear to be visible in a single generation; but the slightest of variation as a result of repeated new association, even though it tend in diverse directions, must in time, guided by natural selection, result in an appreciable difference in a definite direction between a chromosome and its direct descendant and hence between the characters associated with them. In this we have a suggestion of a possible cause of individual variation in homologous chromosomes which we have already seen reason to suspect (pp. 221 and 226).

Finally, we may briefly consider certain observations which seem at first sight to preclude the general applicability of the conclusions here brought out. If it be admitted that the phenomenon of character-reduction discovered by Mendel is the expression of chromosome-reduction, it follows that forms which vary according to Mendel's law must present a reducing division. But the vertebrates and flowering plants—the very forms from which most of the Mendelian results have been obtained—have been repeatedly described as not exhibiting a reducing division. Here, therefore, is a discrepancy of which I venture to indicate a possible explanation in the suggestion first made by Fick[1] and more recently by Montgomery.[2] This is to the effect that in synapsis as it occurs in vertebrates and other forms possessing loop-shaped chromosomes, the union is side by side instead of end-to-end to as in Arthropods. In vertebrates, two parallel longitudinal splits, the forerunners of the two following divisions, appear in the chromosomes of the primary spermatocyte prophases. Both being longitudinal, they have been described as equation divisions, but if it shall be found possible to trace one to the original line of union of the two spermatogonial chromosomes side by side in synapsis, that division must be conceived as a true reduction. A number of observations supporting this view will be brought forward in my forthcoming work on *Brachystola*.

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[1] Fick, R., "Mittheilung ueber Eireifung bei Amphibien," *Supp. Anat. Anz.*, XVI.

[2] Montgomery, T. H., Jr., *loc. cit.*

Again, if the normal course of inheritance depends upon the accurate chromatin-division accomplished by mitosis, it would appear that the interjection, into any part of the germ cycle, of the gross processes of amitosis could result only in a radical deviation from that normal course. Such an occurrence has actually been described by Meves, McGregor and others in the primary spermatogonia of amphibians. In these cases, however, it appears that fission of the cell-body does not necessarily follow amitotic division of the nucleus. I would suggest, therefore, the possibility that the process may be of no significance in inheritance, since by the disappearance of the nuclear membranes in preparation for the first mitotic division, the original condition is restored, and the chromosomes may enter the equatorial plate as if no amitotic process had intervened.

There is one observation in connection with the accessory chromosome which deserves mention in any treatment of the chromosomes as agents in heredity. This element always divides longitudinally and hence probably equationally. It fails to divide in the first maturation mitosis, in which the ordinary chromosomes are divided equationally, but passes entire to one of the resulting cells. In the second maturation division, by which the reduction of the ordinary chromosomes is effected, the accessory divides longitudinally.[2]

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[1] It is of interest in connection with this question that there occurs regularly in each of the spermatogonial generations in *Brachystola* a condition of the nucleus which suggests amitosis but which in reality is nothing more than the enclosure of the different chromosomes in partially separated vesicles. Cf. Sutton, W. S., "The Spermatogonial Divisions in Brachytola Magna," *Kans. Univ. Quart.*, IX., 2.

[2] The chromosome x of *Protenor*, which of all chromosomes in non-orthopteran

My observations in regard to the accessory chromosome lend support to the hypothesis of McClung[1] that of the four spermatozoa arising from a single primary spermatocyte, those two which contain this element enter into the formation of male offspring, while the other two, which receive only ordinary chromosomes take part in the production of females. If this hypothesis be true, then it is plain that in the character of sex the reduction occurs in the first maturation mitosis, since it is this division which separates cells capable of producing only males from those capable of producing only females. Thus we are confronted with the probability that reduction in the field of one character occurs in one of the maturation divisions and that of all the remaining characters in the other division. The significance of such an arrangement, though not easy of perception, is nevertheless great. As regards their chromosome groups, the two cells resulting from each reduction mitosis are conjugates and, therefore, opposites from the standpoint of any individual character. Thus if we consider a hypothetical form having eight chromosomes comprising the paternal series *A*, *B*, *C*, *D* and the maternal series *a*, *b*, *c*, *d*, one of the cells resulting from the reduction division might contain the series *A*, *b*, *c*, *D*, in which case its sister-cell would receive the conjugate series *a*, *B*, *C*, *d*. It is plain that these conjugates, differing from each other in every possible character, represent the most widely different sperms the organism can produce. Now if reduction in the sex-determining chromatin also took place in this division it is apparent that these two diametrically opposite series would enter into individuals of different sexes; but if the sex-reduction is previously accomplished by the asymmetrical distribution of the accessory in the first division, then both the members of each conjugate pair must take part in the production either of males or of females and thus

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forms most closely resemble the accessory, is also described by Montgomery (1901) as dividing in the reducing division, and failing to divide in the equation division—a fact which is the more remarkable because in *Protenor*, as in all Hemiptera-Heteroptera thus far described, reduction is accomplished in the *first* maturation division.

[1] McClung, C. E., "The Accessory Chromosome — Sex Determinant?" BIOL. BULL., III., 1 and 2, 1902. "Notes on the Accessory Chromosome," *Anat. Anz.*, XX., pp. 220–226.

all extremes of chromosome combination are provided for within the limits of each sex.

POSTSCRIPT.

The interesting and important communication of Guyer[1] on "Hybridism and the Germ-Cell" is received too late for consideration in the body of this paper. This investigator also has applied conclusions from cytological data to the explanation of certain phenomena of heredity, and his comparative observations on the spermatogenesis of fertile and infertile hybrids are an important contribution to the cytological study of the subject. The conclusions drawn are of great interest but, I think, in some cases, open to criticism. In assuming that there is a "segregation of maternal and paternal chromosomes into separate cells, which may be considered 'pure' germ-cells containing qualities of only one species" (p. 19), he repeats the error of Cannon which has already been dealt with in the early part of this paper. No mention is made in the paper of Mendel's law but in considering the inbred pigeon hybrids from which his material was obtained, the author expresses his familiarity with manifestations of the Mendelian principle by the statement that "in the third generation there is generally a return to the original colors of the grandparents." In cases which seem to resemble one grandparent in all particulars it is clear that the conception of pure germ-cells may be strictly applied, but the author was familiar with cases of inbred hybrids which plainly show mixtures. These he is inclined to explain in two ways as follows: (1) "Union of two cells representing each of the two original species would yield an offspring of the mixed type." (2) "Besides through the mixing just indicated, variability may be due also in some cases to the not infrequent inequalities in the division of individual chromosomes, through which varying proportions of the chromatin of each species may appear in certain of the mature germ-cells" (p. 20).

The first of these explanations would accord with the result of Mendelian experiment but for the fact that it is erroneously applied (and without cytological grounds) to *all* the characters or chromosomes instead of to individuals. As for the second passage quoted, there can be little doubt that irregular division of chromosomes would be likely to produce marked variation, but as Guyer himself observes, *these irregularities increase with the degree of infertility*. It seems natural to conclude, therefore, that they are not only pathological but perhaps in part the cause of the infertile condition. Furthermore, on the hypothesis of individuality of chromosomes, which Guyer accepts, the loss of a portion of a chromosome by irregular division would be permanent and the effect of repetitions of the operation upon the descendants of a single chromosome group (which he regards as transmitted as a whole) would be so marked a depletion of chromatic substance as must lead soon to malfunction and ultimately to sterility.

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[1] Guyer, M. F., "Hybridism and the Germ-Cell," *Bulletin of the University of Cincinnati*, No. 21, 1902.

As already noted (p. 216) the first of these two explanations of the causes of variation would allow only four possible combinations of chromosomes in the offspring of a single pair. But we know that except in the case of identical twins, duplicates practically never appear in the offspring of a pair however numerous the progeny. Therefore, whatever the number of the offspring, the variations of all except the few provided for by the four normal chromosome combinations must be accounted for by obviously pathological division processes, which tend strongly in the direction of sterility. But in the report of Bateson and Saunders to the Evolution Committee we find the statement: "We know no Mendelian case in which fertility is impaired" (p. 148). When we reflect that the vast majority of cases studied by these observers were Mendelian and connect this piece of evidence with the testimony of Cannon[1] that the maturation processes of variable cotton-hybrids are either normal or so distinctly abnormal as to entail sterility and with Guyer's own admission that the abnormalities in mitosis increase with the degree of sterility, the balance is strongly against the efficacy of pathological mitoses as factors in normal hybrid variation.

I take pleasure in acknowledging my indebtedness to Professor E. B. Wilson for invaluable counsel in the presentation of a subject offering many difficulties.

Department of Zoölogy, Columbia University,
January 25, 1903.

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[1] Cannon, W. A., *loc. cit.*

ON PHYLLODISTOMUM AMERICANUM (N. SP.); A NEW BLADDER DISTOME FROM AMBLYSTOMA PUNCTATUM.

HENRY LESLIE OSBORN.

Part I.

The species of this genus which have been reported hitherto have all come from the old world. *P. folium* Olf., from Europe, and *P. patellare*, Sturgis, '97, from Japan, have been till recently the only species known; during 1902, however, Odhner added

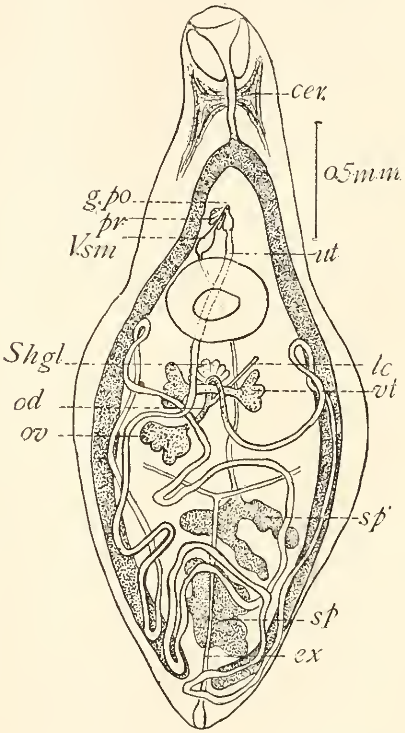


Fig. 1. Ventral view of *P. americanum*, camera lucida, × 32 diam. *g.po.*, genital pore; *ut.*, uterus; *l. c.*, Laurer's canal; *vt.*, vitellaria; *V. def.*, vas deferens; *sp.'* anterior testis; *sp.''*, posterior testis; *ex.*, excretory bladder; *int.*, intestine; *ov.*, ovary; *Sh.gl.*, shell gland; *V.sm.*, seminal vesicle; *pr.*, prostate cells.

four species from fishes of northeastern Africa. I have been greatly interested to find that some of the salamanders of this neighborhood, *Amblystoma tigrinum*, are infected with a species of *Phyllodistomum* unlike any hitherto reported. Pending a later fuller account of the structure of the fluke, this brief notice will indicate the chief points in its anatomy.

The worm is rather uncommon. In twenty-nine salamanders that have been examined for it it has been found in only six cases.

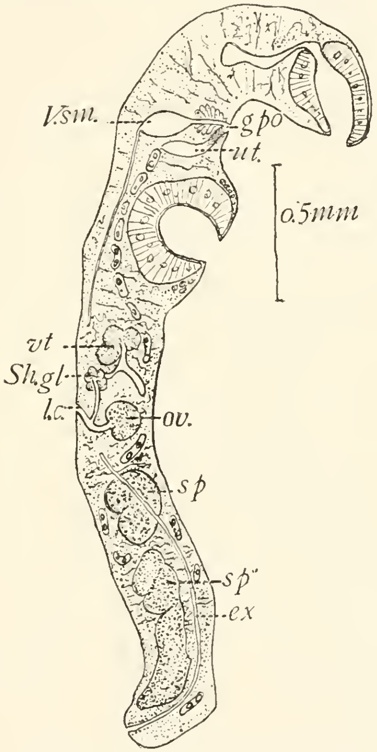


FIG. 2. Sagittal section, × 36. Camera lucida ; lettering as in Fig. 1.

The parasite is found in the urinary bladder of the salamander. In one case nineteen flukes were found, but this was exceptional, for two, three or four mature flukes is the maximum found in the other cases, beside perhaps two or three small flukes apparently a young stage of the others. The coarser features of the organization are shown in Fig. 1, a ventral view from a specimen preserved (without compression) in corrosive sublimate solution and stained, cleared and mounted whole. The form of the body is less spatulate than that of *P. folium* or *P. patellare*, the neck is less distinct. The total length of this specimen is 3.5 mm., its greatest breadth is 1.4 mm., the ratio of breadth to length is thus 40 per cent. In *P. patellare* this ratio is 66 per cent., in *P. spatula* (Odhner, '02) it is 48 per cent. The American form is thus less broad than any reported old world form. There is considerable variation in this respect in my material, some cases being decidedly slender, in one mature specimen studied alive under some compression and measured from the camera lucida drawing, the length is 4.2 mm., the width 0.88 and the ratio of breadth to length 20.9 per cent. This specimen is more than usually narrow, most having the broadened form of Fig. 1.

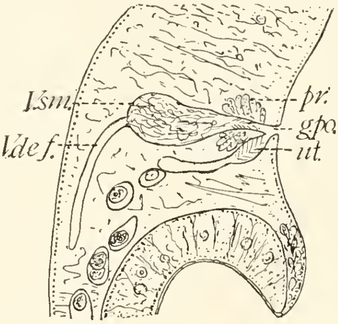


FIG. 3. Section at the genital pore, × about 120.

There is no pharynx, the œsophagus is short, the intestines branch well forward, and are very long, reaching to the extreme posterior end of the body. The cells of the intestine are provided with very long cilia, as in *P. patellare;* they would probably be very interesting subjects for histological study. In both *P. folium* and *P. patellare* the intestines are somewhat sacculated, but here they are entirely simple.

The excretory system is like that of *P. folium* and *P. patellare* — dorsal posterior terminal pore, a long ventrally located bladder branching anteriorly on the level of the hinder boundary of the ovary. Smaller vessels and flame cells like Fig. 5 of Sturgis, '97, are recognizable in sections.

The chief important internal differences between this species and those previously known are in the reproductive system. The two testes are both nearly in the middle line, the anterior slightly more on the left side, both are entirely within the hinder third of the body. The anterior testis lies wholly posterior to the ovary, unlike either *P. folium* where it lies on the same level as the ovary, or *P. patellare* where a considerable part is anterior to the ovary. Both testes are very deeply and irregularly lobed, but they are not divided. The lobing is deeper than in any other species of the genus, in some of which (*e. g.*, *P. unicum*, Odhner, '02) they are entire. The cirrhus organ is present, not enveloped in a sac, there is a small seminal vesicle, the ductus ejaculatorius is ciliated and surrounded by prostate cells not marked off from the parenchyma by a membrane. The genital pore is situated in the middle line some distance in front of the ventral sucker.

The ovary is located on the right side and in front of the anterior testis (usually, but on the left side in occasional instances). It is deeply lobed. The oviduct arises from its dorsal surface (see Fig. 4) and passes toward the anterior end. A passage (Laurer's canal) is given off from the oviduct and runs to the dorsal surface and right side and there opens to the exterior. There is no seminal receptacle. The oviduct running on passes through a distinct shell gland, receiving at this place also the duct from the vitellaria; it then passes on as the uterus arching

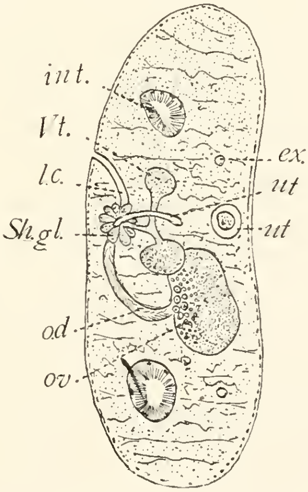


FIG. 4. Combined view from several serial sections showing the relation of the ovary and the neighboring parts, all represented as if on the same level, × about 120.

over the vitelline duct and passing backward toward the posterior end of the body. The course of the uterus is unlike that of any described member of this genus, and is shown in detail in Fig. 1, where however the coils are somewhat simplified for the sake of clearness. The course is first a forward loop on the left side, then on the same side a loop running to the hind end of the body, having on its return part a side loop down into the space between the two testes, then another loop across the front of the anterior testis, then the uterus crosses to the right side and forms first an anterior loop and finally a long posterior one, at last passing across in front of the ovary and ventrally to and between the two vitella and then dorsally over the ventral sucker to reach the genital pore. There is hardly any atrium (Fig. 3), the male and female ducts meeting almost as they reach the surface. The terminal part of the uterus is ciliated like that of the ductus ejaculatorius. The ova measure 0.052 mm. x 0.050 mm.

The vitellaria lie close together near the center of the body. They are lobed, each consisting of about three parts, one in the center and one in front of this and one behind it. These are not separate follicles, but lobes of a single organ.

Of the generic identity of this form with *Phyllodistomum* of Braun, '99, there can be no doubt. The specific distinctness is equally clear. As it is the first form to be reported from this country I propose the name *americanum* to designate the species.

PART II.

Since the foregoing part of this article was written an article has been published by Stafford, '02, on the American Representatives of *Distomum cygnoides*, in which a new form is described under the name of *Gorgodera translucida*, which bears a considerable resemblance to *P. americanum*. I have also had an opportunity which I owe to the kindness of Professor Stafford of examining some specimens of the species he has described.

The transparency so noticeable in Stafford's form is also noticeable in some of my specimens. My material is derived from two sources, first from worms found by an assistant (Mr. C. C. Tyrrell) in the laboratory and at once (except one studied alive) placed in corrosive sublimate solution, which are all opaque, and specimens found by opening the bladders of salamanders which had been preserved in formaline for anatomical work. These latter were, many of them, quite decidedly translucent. I think it likely that as soon as I can obtain living specimens I shall find them at least somewhat translucent if less so than Stafford's.

The form of the body in *G. translucida* is slender and parallel-sided, and not at all inclined to a spatulate form. While this is quite unlike the form of Fig. 1, there are among my specimens some in which the body is very slender. I have not yet decided to what extent this may be due to the youth of the specimen. Young worms found in bladders with mature specimens and apparently earlier forms of this species are decidedly slender in outline and not spatulate. I have even found some in which eggs were already filling the uterus in which this elongate form was still psesent. I have little doubt that fully mature older specimens all become spatulate eventually. These facts indicate a gradual shading from one to the other of these contrasted body forms.

There appears also to be considerable similarity in the arrangement of the coils of the uterus in these two forms.

The location of the ventral sucker is much farther forward in *G. translucida* than in *P. americanum*, being in the former 20 per cent. of the total length from the anterior end and in the latter 32–42 per cent. The testes are located in the hinder third of the body and are deeply lobed in *P. americanum*, and are in the middle third and are nearly entire in *G. translucida*. The ovary is behind the middle of the body not near the ventral sucker and is deeply lobed in *P. americanum*, and is in front of the middle of the body near the ventral sucker and entire in *G. translucida*.

These differences are not entitled to be rated as of sufficient value to justify placing these forms in different genera, unless the body form proves to be a difference of more importance than at present appears. It is the only character that is offered by Looss, '99, by which to distinguish his *Spathidium* (= *Phyllodistomum* of Braun, '99) from *Gorgodera*, though some species of the latter (*e. g.*, *G. cygnoides*) differ in having many testes instead of a single pair. For the present and until more is known of *P. americanum* the spatulate form will have to remain the distinguishing mark of the genus. Should its rank as a critical feature be lost, it seems that we should then be obliged to bring *Gorgodera translucida*, and with it some other species of the genus into the genus *Phyllodistomum*.

HAMLINE UNIVERSITY, ST. PAUL, MINN.,
February 4, 1903.

LITERATURE.

Braun, M.
'99 Ueber Clinostomum Leidy. Zoöl. Anz., XXII.

Looss, A.
'94 Die Distomen Fische u. Frosche. Bibl. Zoöl., XVI.

Looss, A.
'99 Weitere Beitræge zur Kentn. Trem-Fauna Ægyptens. Zoöl. Jahrb. Syst., XII.

Odhner, Th.
'02 Mitt. z. Kentn. Distom. II. Zur. Kent. Harnblase-distomen der Fische. Cent. f. Bakt. u. Parasit., XXXI.

Stafford, J.
'02 The American Representatives of Distomum cygnoides. Zoöl. Jahrb. Syst., XVII., p. 411.

Sturgis, M. M.
'97 Preliminary notes on Distomum patellare, n. sp. Zoöl. Bulletin, I., p. 57.

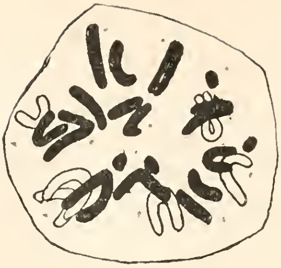
THE HETEROTYPIC MATURATION MITOSIS IN AMPHIBIA AND ITS GENERAL SIGNIFICANCE.

THOS. H. MONTGOMERY, JR.

The marked contradiction between the results of workers on spermatogenesis of vertebrates, and of those on the spermatogenesis of arthropods, has led me to examine the formation of the chromosomes of the first maturation mitosis in urodele amphibia. The question at issue is of course the interpretation of the heterotypic division, for if that is an equational division as generally maintained, then in vertebrates there would be no transverse, or so-called "reduction," division of the chromosomes. Much time and thought has been given to the interpretation of the various ring-shaped chromosomes of heterotypic divisions, *i. e.*, as to whether the space enclosed by the ring represents a longitudinal split of a single chromosome, or a space between two univalent chromosomes. Uncertainty and confusion has resulted, because most of these interpretations have not taken into account the earliest stages in the formation of such chromosomes, which are really the only stages that need critical examination.

The two species in which these decisive periods of the spermatogenesis was studied, are *Plethodon cinereus* (Green) and *Desmognathus fuscus* (Raf.); the maturation mitoses occur in the summer, and the testes were fixed in Hermann's and Flemming's solutions, and stained with iron hæmatoxyline. The spermatogenesis is essentially alike in both; Figs. 1–6 are camera drawings of spermatocytes of *Desmognathus*, and 7 and 8 of *Plethodon*.

The normal number of chromosomes is twenty-four as shown clearly on a pole view of a monaster (equatorial plate) stage (Fig. 1); in this figure four of the chromosomes are cross-sectioned, and one of the others shows very clearly the longitudinal split. In the spermatocytes which result from the last spermatogonic division there is just half this number of chromosomes, namely twelve, the so-called reduction in number taking place



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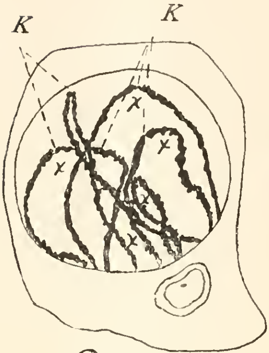

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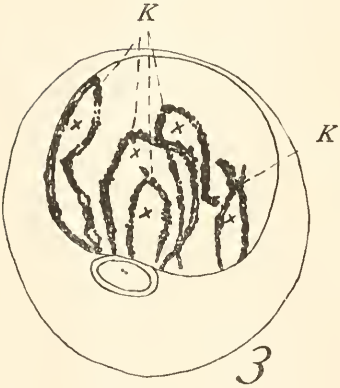

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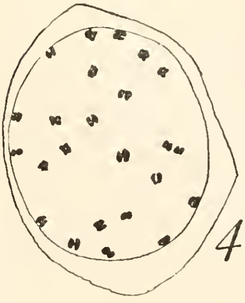

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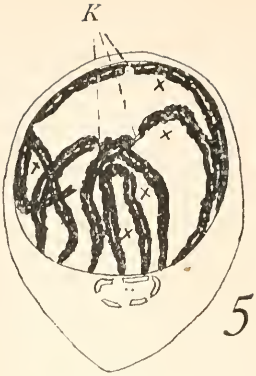

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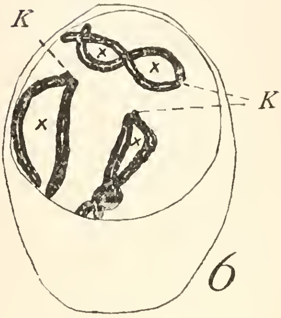

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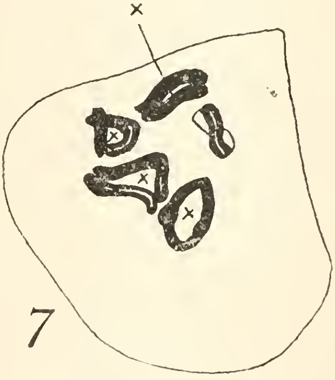

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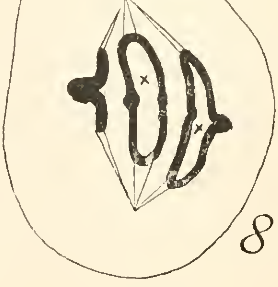

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in the synapsis stage of the growth period before the maturation divisions. Now what has to be determined is the changes that occur in these chromosomes in the prophases of the first maturation mitosis. In the early prophases (Figs. 2, 3, 5) these chromosomes always show a definite arrangement; in the figures only a few of the chromosomes are shown in each case, namely those which are seen in their entirety in the plane of the section. There is a distinct polarity of cell body and of chromosomes, and it is the same polarity which I found to obtain in the spermatocytes of *Peripatus*: the nucleus lies in the portion of the cell where there is the least amount of protoplasm, and the sphere (idiozome body) at that side of the nucleus directed towards the greatest protoplasmic mass. For these two poles, as a translation of Rabl's "Pol" and "Gegenpol," I used in the case of *Peripatus* the terms "central pole" and "distal pole," and these terms may equally well be applied to the amphibian spermatocytes. The arrangement in the case of the chromosomes is well shown in the Figs 2, 3 and 5. Each chromosome has the form of a loop like a U or a V with the bend or angle of the chromosome pointing towards the central pole, and the free ends terminating at the distal pole of the nucleus; sometimes at these early stages the two free ends of a chromosome may be applied together so that the whole chromosome has the form of an elongated ring; but generally in these early prophases the U or V shape is the prevalent one.

There are just twelve of these looped chromosomes, half the number of those in the spermatogonia, as may be determined by study of cells cut in a plane at right angles to the axis connecting the central and distal poles. Thus in Fig. 4 (corresponding to the stage of Fig. 3) can be counted twenty-four cross-sections of chromatin threads, every two of which represent the two arms of one of the twelve looped chromosomes. Now the point of great importance is that these early U and V-shaped chromosomes have not arisen by any longitudinal splitting of a single chromosome, for in the very earliest prophases, even earlier than that represented in Fig. 2, they have this shape; therefore the space circumscribed by the two arms of the chromosome does not represent a longitudinal split, but a longitudinal split appears later and then along the axis of each arm (Figs. 3–6). What then is the correct interpretation of each chromosome loop? Each U or V is, first of all, a bivalent chromosome, since they occur in half the number of the chromosomes of the spermatogonia. Second, each arm of one of these bivalent chromosomes represents one of the univalent chromosomes of the spermatogonia. In the case of a bivalent chromosome of the form of a U or V, the two univalent chromosomes are connected together at the angle, that is one end of one chromosome is joined to one end of another chromosome; these points of union are marked in the Figs. 2, 3, 5 and 6 by the letter *K*. Just at this point of junction can be seen in many cases, though not so clearly as in *Peripatus*, a broad connecting linin thread, as in the chromosomes of Fig. 5. When a bivalent chromosome is an elongate ring, as in the case of the left-hand one of Fig. 3, then both ends of both univalent chromosomes are connected. The bivalent chromosomes of *Desmognathus* and *Plethodon* thus represent each two univalent chromosomes joined end to end, and the space between the two arms of a bivalent chromosome is the space between two univalent chromosomes, whether this space be bounded by a chromosome of the form of a U, a V, or an O. This is the space marked in the figures by the letter x, and, as the present observations show, does not represent a longitudinal split since it is widest at the earliest stages of the prophase.

The longitudinal splitting of the chromosomes is shown in its commencement in Fig. 3, where certain microsome groups are splitting ahead of others. Fig. 5 shows the stage where this split is most pronounced. Fig. 4 shows the split on cross-sectioned chromosomes. It is still apparent in some of the chromosomes of Fig. 7, but in the equatorial plate stage (Fig. 8) is hidden, to reappear at the anaphase as a longitudinal split of each of the daughter (univalent) chromosomes. This temporary disappearance of the longitudinal split during the equatorial plate stage of the first maturation mitosis has been noted in various objects, and I have described it in detail for *Peripatus* and the *Hemiptera*, as has Korschelt for *Ophryotrocha*. Along the plane of this split the daughter chromosomes divide in the second maturation mitosis, so that the latter is an equation division.

It is then apparent that the split along each arm of a bivalent chromosome, is a longitudinal split of each univalent chromosome, and is a preparation for the second maturation mitosis. It is equally evident that the space marked *x* in the figures does not represent any longitudinal split, but a space between two univalent chromosomes. Hence in the typical chromosomes of the ring form, as shown in Fig. 8, the space enclosed by the chromosome is the space between two univalent chromosomes, and has nothing to do with the longitudinal split. The thickenings so frequently found upon the rings, as in Figs. 7 and 8, are the points of union of the ends of two univalent chromosomes, as becomes clear from the intermediate stages shown in Figs. 6 and 7, and are not, as generally interpreted, portions of the chromosome where the longitudinal split is least evident. The reader may follow the spaces marked *x* in the chromosomes from the stage of Fig. 8 back through successive stages to Fig. 2, to be certain of this fact. And it is decisive that this space becomes wider the earlier the prophase, as a comparison of Figs. 8 and 2 shows, and does not lead back to a simple longitudinal splitting.

So the form of the bivalent chromosomes is explained, and the fact established that the heterotypic mitosis, the first maturation mitosis, is not an equation division but separates entire univalent chromosomes, while the second maturation mitosis is equational.

These views are in disagreement with the conclusions of most other workers on amphibian spermatogenesis, because the writers have mostly assumed that the space marked *x* in my figures, the space enclosed by the ring, is a longitudinal split. Flemming first described the heterotypic mitosis and named it; he overlooked the true longitudinal split in each arm of a bivalent chromosome, and concludes the space enclosed by the definitive chromosome to be the longitudinal split. Vom Rath described in *Salamandra* a reduction division on the basis of tetrad formation with spherical chromosomes, but later observers have demonstrated that he studied abnormal mitoses. Meves essentially corroborates Flemming's interpretations, but he did not note the polarity of the spermatocytes as evidenced in the arrangement of the chromosomes, though it is shown in his Fig. 47, Pl. IV., and hence leaves untouched the question of the origin of the bivalent chromosomes; he also failed to describe the true longitudinal split in each arm of the chromosomes, though certain of his figures show it indistinctly. McGregor confirmed for *Amphiuma* Meves' account for *Salamandra*, but likewise failed to show how the bivalent chromosomes are produced; his Fig. 7, Pl. IV., shows the true longitudinal split, but it is not demonstrated by his figures that this becomes the space enclosed by the definitive chromosome. Eisen likewise interprets, for *Batrachoseps*, the heterotypic division as an equation division, though his "bouquet stage" is essentially similar in its details to my Fig. 2, and though his results might equally well be interpreted as speaking for a transverse division. Kingsbury's account for *Desmognathus* is clearly a careful study; his Figs. 4 and 5 may be compared with my Figs. 4 and 3; he found the polarity of the chromosomes in the nucleus, saw the true longitudinal split (his Fig. 6), but illustrates no stages to show that this split is connected with the space in the definitive chromosomes. Janssen's account for *Triton* appears to be the most detailed and careful, his Figs. 4, 5 and 32 show appearances of the early chromosomes just as I have found; he describes the synapsis stage where the formation of bivalent chromosomes takes place; he was the first to clearly recognize this stage in the Amphibia, but holds the bivalent chromosomes undergo two longitudinal splittings after their transverse segmentation from a continuous chromatin spirem.

In the ovogenesis of Amphibia, to mention only two more recent studies, Lebrun concludes that both maturation divisions are equational, though more on the basis of a lengthy discussion of the definitive forms of the chromosomes than of a description of the early formation stages. Miss King, for *Bufo*, was unable to determine whether the heterotypic division is transverse or equational.

The whole question is one of a careful interpretation of the early stages of the chromosomes, and the mode of formation of the bivalent chromosomes. The workers on this subject have been satisfied for the most part to know that in the spermatocytes the chromosomes are bivalent, without describing the mode of union of every two univalent chromosomes. In *Desmognathus* there is one true longitudinal split, and besides that what has heretofore been regarded as a second longitudinal split but what is really the space between the two univalent chromosomes of a pair.

The term "reduction division" was introduced by Weismann, to denote a division that separates entire chromosomes, in contradiction to "equation division" which halves longitudinally each chromosome. Weismann, in his splendid conclusion that such a division would be found to occur, unfortunately assumed that there must be a doubling of the normal number of chromosomes before the maturation. This doubling does not occur, as Boveri and Brauer were the first to demonstrate, but instead there is in the synapsis stage, as I first showed, a union of chromosomes end to end in pairs. This union is not due, as Rückert suggested, to the chromatin spirem breaking into half the somatic number of chromosomes, since there is no continuous *chromatin* spirem in the prophases of the maturation mitosis in any of the objects studied by me, but is a union of chromosomes that were disunited before.

That a separation of entire univalent chromosomes in one of the maturation divisions does occur, is shown by the following studies: Rückert and Häcker for the ovogenesis of Copepoda, finding the separation of entire univalent chromosomes to occur in the *second* division; while Lerat does not decide whether such a division occurs, but points out that it is the *first* division which is heterotypical. In the spermatogenesis of *Gryllotalpa*, by Vom Rath; of Hemiptera, by Henking, myself and Paulmier; of Orthoptera by McClung, Sutton, and Wilcox. The first maturation in the ovogenesis of *Ophryotrocha*, according to Korschelt's account, also separates entire univalent chromosomes, and very clearly the first maturation in the spermatogenesis of *Peripatus* according to my own observations. For the ovogenesis of *Limnæa* this is maintained by Linville, but the chromosomes in this object are unfavorable for decisive interpretation. In Isopoda (*Oniscus*) the first division was shown to be reductional by Miss Nichols. For *Thalassema* and *Zirphæa* the same conclusions were reached by Griffin; and Lillie considers it possible that Griffin's mode of interpretation may be applied to the egg of *Unio*. Van Winiwarter in a very excellent detailed study of these stages in the ovogenesis of mammals (*Homo*, *Lepus*), considers it probable that one of these divisions is reductional. Schockaert, for the ovogenesis of *Thysanozoön*, finds also a reduction division, in contradiction to the earlier work of Van der Stricht. Finally, Carnoy's earlier work speaks very strongly for the occurrence of reduction divisions.

It is then very humorous to read in a recent paper by Meves: "Ich für meine Person halte es aber, heute mehr als je, für gerechtfertigt, das Vorkommen sogenannte Reduktionsteilungen zu bezweifeln." Herr Meves has the right to hold any opinion he pleases, but he will soon find himself in the small minority where dogmatic assertion takes the place of fair-mindedness. As the pupil of Flemming, Meves must stand by his teacher, to the effect that there occur only equational divisions. Herr Meves has had very little experience in the field of the phenomena of maturation, though he has done excellent work in the study of the formation of the spermatozoön, and in his paper on the spermatogenesis of *Salamandra* overlooked all the phenomena which are decisive in regard to a reduction division. I might add, "ich für meine Person halte Herrn Meves' Resultate unbewiesen zu sein."

In the light of the recent work it becomes apparent that "heterotypic" division is synonymous with "reduction" division; it is heterotypic in being the only mitosis in the germinal cycle in which entire univalent chromosomes are separated in metakinesis. This explains why the shape of the chromosomes in this mitosis differ from all other mitoses; if it were an equation division, why should its chromosomes differ so markedly from those in other equation divisions? "Transverse," "reduction" and "heterotypic" divisions express the same phenomenon in different words, and the nail is driven home when the facts are so clear in amphibian spermatogenesis, in the very cells which were held to disprove the occurrence of reduction mitoses.

In my paper on *Peripatus* I proved that the bivalent chromosomes are produced by the union end to end of every two univalent chromosomes, and not by a splitting of a chromatin spirem into half the normal number of segments. This has since been found by several observers to be the case for other objects. Then in a paper, "The Germ Cells of the Metazoa," I showed that each bivalent chromosome is very probably formed by the union of a paternal with a maternal chromosome, and interpreted this process as part of the phenomenon of conjugation, as a conjugation of the chromosomes. I showed also that when in the spermatogonia certain chromosomes can be distinguished from the others by peculiarities of size, that such chromosomes pair together in the synapsis; this was the case in *Protenor*, *Peliopelta* and *Zaitha*. McClung (1902) has noted a similar case for *Anabrus*. A few months ago Sutton confirmed these conclusions for *Brachystola*, and on the basis of a careful examination of the chromosomes has reached the important conclusion, that, maternal chromosomes of a certain length uniting always with paternal chromosomes of the same length, the result of the reduction division, in separating such two univalent chromosomes from each other, prevents the retention by either of the daughter cells of two chromosomes representing the same characters. Such a result is a strong confirmation of the brilliant postulates of Weismann.

If it be true that wherever a heterotypic mitosis occurs, it denotes the separation of entire univalent chromosomes, then the old strife of whether a reduction division does or does not take place in the maturation of the germ cells is decided in the affirmative, and we are no longer met by the discrepancy of certain animals with and certain without a reduction division. It rests with the opponents of this view to prove that the heterotypic division is an equation division, and that has not yet been satisfactorily done. And in reaching this conclusion I may state frankly that at the outset of my studies I was fully convinced, almost as much as Herr Meves himself, that the heterotypic mitosis is an equation division; only long observations have shown me that it can only be regarded as a reduction division.

McClung has recently (1902) made an embittered attack upon my studies on spermatogenesis, due in large part to a misunderstanding of my position. He states at various places that my views are conflicting, and that he is unable to harmonize them. Certain serious mistakes I made in my first paper (1898) I took pains to correct in two others (1899, 1901), and in these my position is stated very definitely and without contradictions.

Were he as frank in admitting mistakes, there would be great unanimity.

LITERATURE CITED.

Boveri.

Zellen-Studien, 2. Jena, 1888.

Brauer.

Zur Kenntniss der Spermatogenese vom Ascaris megalocephala. Arch. f. mikr. Anat., 42, 1893.

Carnoy.

La cytodiérèse chez les Arthropodes. La Cellule, 1, 1885.

Eisen.

The spermatogenesis of Batrachoseps. Journ. Morph., 17, 1900.

Flemming.

Neue Beiträge zur Kenntniss der Zelle. Arch. f. mikr. Anat., 29, 1887.

Griffin.

Studies on the Maturation, Fertilization, and Cleavage of Thalassema and Zirphæa. Journ. Morph., 15, 1899.

Häcker.

Die Vorstadien der Eireifung. Arch. f. mikr. Anat., 45, 1895.

Henking.

Ueber Spermatogenese und deren Beziehung zur Eientwicklung bei Pyrrhocoris apterus M. Zeitschr. wiss. Zool., 51, 1890.

Janssens.

La Spermatogénèse chez les Tritons. La Cellule, 19, 1901.

King.

The Maturation and Fertilization of the Egg of Bufo lentiginosus. Journ. Morph., 17, 1901.

Kingsbury.

The Spermatogenesis of Desmognathus fusca. Amer. Journ. Anat., 1, 1902.

Korschelt.

Ueber Kerntheilung und Befruchtung bei Ophryotrocha puerilis. Zeitschr. wiss. Zool., 60, 1895.

Lebrun.

La Cytodiérèse de l'œuf. La Cellule, 19, 20, 1901.

Lerat.

La première cinèse de maturation dans l'ovogénèse et la spermatogénèse du Cyclops strenuus. Anat. Anz., 21, 1902.

Lillie.

The Organization of the Egg of Unio, etc. Journ. Morph., 17, 1901.

Linville.

Maturation and Fertilization in Pulmonate Gasteropods. Bull. Mus. Comp. Zoöl., Harvard, 35, 1900.

McClung.

The Spermatocyte Divisions of the Locustidæ. Kansas Univ. Sci. Bull., 1, 1902.

McClung.

The Spermatocyte Divisions of the Acrididæ. Ibid., 1900.

McGregor.

The Spermatogenesis of Amphiuma. Journ. Morph., 15, 1899.

Meves.
Ueber die Entwicklung der männlichen Geschlechtszellen von Salamandra maculosa. Arch. f. mikr. Anat., 48, 1896.

Meves.
Über oligopyrene und apyrene Spermien, etc. Ibid., 61, 1902.

Montgomery.
The Spermatogenesis in Pentatoma up to the Formation of the Spermatid. Zool. Jahrb., 12, 1898.

Montgomery.
Chromatin Reduction in the Hemiptera, a correction. Zool. Anz., 22, 1899.

Montgomery.
The Spermatogenesis of Peripatus (Peripatopsis) balfouri up to the Formation of the Spermatid. Zoöl. Jahrb., 14, 1900.

Montgomery.
A Study of the Chromosomes of the Germ Cells of Metazoa. Trans. Amer. Phil. Soc., 20, 1901.

Nichols.
The Spermatogenesis of Oniscus asellus Linn. Proc. Amer. Phil. Soc., 41, 1902.

Paulmier.
The Spermatogenesis of Anasa tristis. Journ. Morph., 15, 1899.

Rabl.
Ueber Zelltheilung. Morph. Jahrb., 10, 1885.

Ruckert.
Zur Eireifung bei Copepoden. Anat. Hefte, 8, 1892.

Schockært.
La ovogénèse chez le Thysanozoon brocchi. La Cellule, 18, 20, 1901, 1902.

Sutton.
On the Morphology of the Chromosome Group in Brachystola magna. Biolog. Bull., 4, 1902.

Van der Stricht.
La formation des deux globules polaires, etc., dans l'œuf de Thysanozoon Brocchi. Arch. de Biol., 15, 1898.

Vom Rath.
Zur Kenntniss der Spermatogenese vom Gryllotalpa vulgaris Latr. Arch. f. mikr. Anat., 40, 1892.

Vom Rath.
Beiträge zur Kenntniss der Spermatogenese vom Salamandra maculosa. Zeitschr. f. wiss. Zoöl., 57, 1893.

Wilcox.
Spermatogenesis of Caloptenus femur-rubrum und Cicada tibicen. Bull. Mus. Comp. Zoöl. Harvard, 27, 1895.

Wilcox.
Further Studies on the Spermatogenesis of Caloptenus femur-rubrum. Ibid., 29, 1896.

Winiwarter.
Recherches sur l'Ovogenèse et l'Organogenèse de l'Ovaire des Mammifères (Lapin et Homme). Arch. de Biol., 17, 1900.

University of Pennsylvania, Philadelphia,
February 10, 1902.

AN OUTLINE OF THE DEVELOPMENT OF A CHIMÆROID.

BASHFORD DEAN.

The common chimæroid of the west coast of the United States, *Chimæra colliei* Jenyns, was taken in deeper water (50–150 fathoms, sp. gr. 1.027, 55° F.) off the Californian coast and in shallower (5–20 fathoms) in Puget Sound. Near Monterey, in water of about 100 fathoms, eggs about to be deposited were taken from females and incubated in sunken cases; by this means a fairly representative series of embryonic stages was secured.[1] In addition one embryo was obtained from an egg-case taken accidentally on the hook of a trawl line, and a series of hatched young were kindly placed at the writer's disposal by the U. S. National Museum.

Spawning occurs at Monterey throughout spring, summer and fall, and a few eggs were obtained by the Chinese fisherman Ah Tack Lee during the winter. The period of maximum spawning is during August. Two eggs are deposited at the same time. And for several hours at least, at the time of protrusion, they hang freely in the water, the small end of each egg-case attached close to the genital opening. The case here terminates in a single string-like process which passes up the oviduct as far as the capsular gland, and here it is so firmly attached at this stage that it can be lengthened — to the degree of several inches — and shortened like a strand of gutta-percha. Exactly how the egg is deposited is unknown; in any event it appears to be at first attached to stones, etc., by a bulbous tip at the end of the string-like process of the egg-case.

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[1] The writer is greatly indebted to President Jordan and to the directors of the Hopkins Seaside Laboratory, at Pacific Grove, for many courtesies extended him during two summers at the laboratory; and to Dr. Ray L. Wilbur for much generous and skilful coöperation in securing material from the Chinese fisher-people during the years 1897, 1898 and 1899. Dr. Wilbur made numerous trips from San Francisco to Monterey during this time, and to his interest in my work and to his boundless energy I am indebted for many of the later and rarer stages of this interesting fish. To Ah Tack Lee, most skilful and intelligent of local fishermen, I owe my best thanks for his services as a collector. To Mr. Naohidé Yatsu, finally, for much valuable assistance in connection with the preparation of the present paper. His are the drawings from which Figs. 8–12, 17–19 are reproduced.

Copulation takes place shortly before the eggs are deposited, for females with eggs in oviducts are usually found with recent marks of the prehensile organs of the male. That the male twists about the female, shark-like, is evident from the character and nearly uniform position of scars near the base of the dorsal fin of the female. These markings, corresponding with the denticles of the frontal organ, indicate that the pair are locked together in copulo. Both mixipterygia appear to be inserted.

There is evidence from experiments with gravid fish kept in aquaria that the elaborate egg-case (Fig. 1) takes but a short time to be formed, possibly not longer than three days. The dilated portion of the case is laid down and is almost perfect, including the lateral processes before the stalk appears; during this time the lining of the oviduct becomes curiously developed to produce the highly specialized structures of the case. The exit-slit or "door" is formed by an abrupt folding in the case's wall. In this folding there is a double row of transversely-directed, interlocking

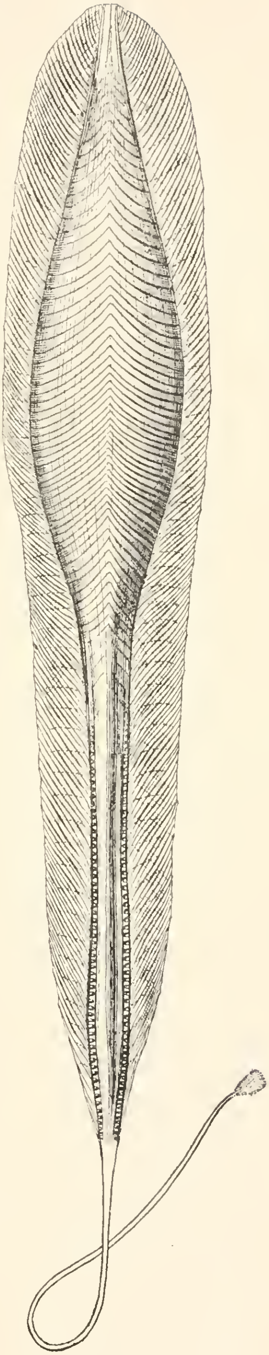
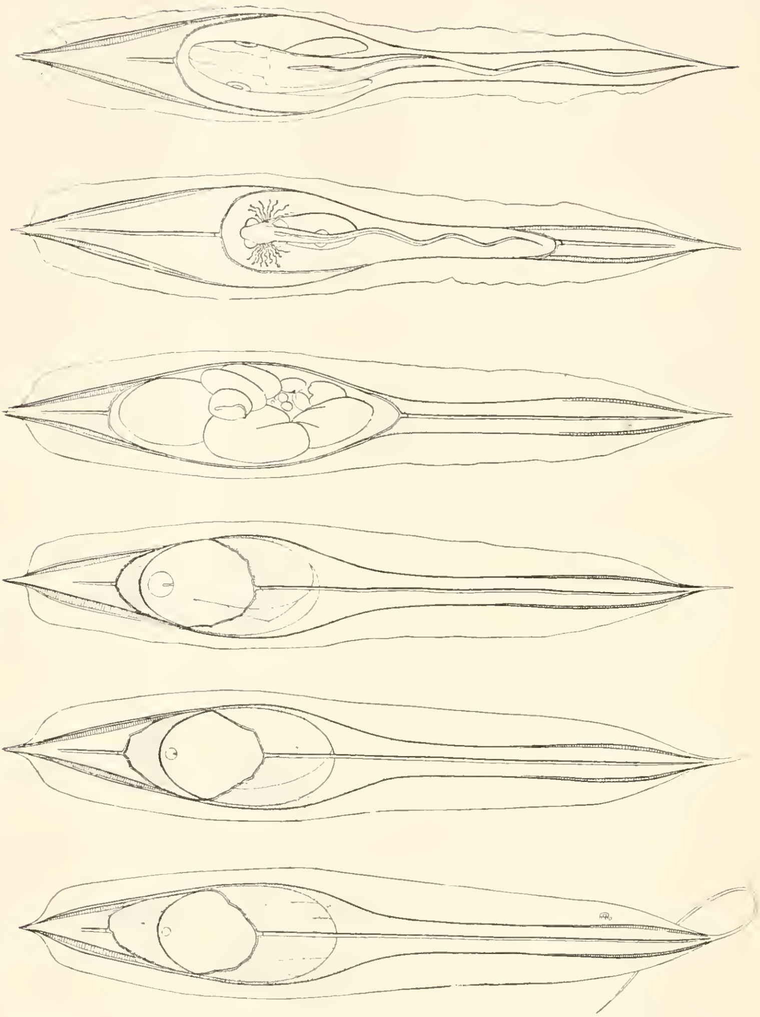


FIG. 1.

FIG. 1. Egg-case of *Chimæra colliei*. 'Ventral' aspect. × 1. The case is of extraordinary length (about seven inches to the base of the terminal filament) compared with the total length of the female (about twenty-three inches). The egg itself when freshly deposited is elongate and depressed (about 1⅛ in. x ¾ x ½ inches), after the fashion of elasmobranchs. It is of similar syrupy consistency, flowing away as soon as the vitellina is ruptured.



Figs. 2–7. Characteristic stages in the development of *Chimæra*. These are shown in the opened egg-capsules and represent (2) late blastula, (3) gastrula, (4, 5) two stages of early embryos, (6) a late embryo, and (7) a young fish about to escape from the capsule. The retarded segmentation of the egg is indicated in Figs. 2–5, but in the three earlier stages the cleavage lines cannot be seen through the wall of the egg-capsule (as has been indicated diagrammatically).

lamellæ, and around their delicate tips the thin walls of the shell weather away so that, by the time of hatching, the interlocking[1] lamellæ can disengage and thus permit the young fish to push its way through the folds. The exit-slit of the egg-case is thus a line of rupture. By somewhat similar lamellæ framing a bilateral series of fenestræ ventilation within the case is obtained and perfected in later stages. The fenestræ (as in eggs of certain elasmobranchs) occur in the hinder end of the case, and in latest stages water flows through them, a current being created by the constantly undulating tail of the embryo. The latter has at all times a highly specialized relation to the egg-case, as is indicated in Figs. 2–7. As soon as the embryo can be determined its head points toward the opening-end of the case, the keeled side of the case being dorsal. It is evident that the narrow end becomes the neatly fitted sheath for the elongated tail, and that the widened end fits accurately the greatly enlarged head and trunk. The young hatches as soon as the straight egg-case is filled; thus there is no coiling and wrapping up of the later embryos, as is found, for example, in the case of many sharks.

The Duration of Development.—After the egg is deposited (water temperature 50°–60° F.) fertilization stages continue for about twenty-six hours. If, therefore, we assume that the formation of the egg-case began with fertilization the total duration of this process is probably not less than three days, and not longer than five.

Segmentation (of nuclei), beginning about twenty-six hours after the egg is deposited, lasts from three to four days, each earlier cleavage taking about two hours.

A well-marked blastula is 110 hours old (Fig. 2).

Gastrulation dates from about the eighth day. On the tenth day the embryo resembles Balfour's stage *C* (*Pristiurus*) (Fig. 3); on the seventeenth, *D*; on the nineteenth, *E*; and on the twenty-second, *F* (Fig. 4).

Of later embryos one resembling stage *I* is thirty-one days old (Fig. 5). I have no accurate data of older stages. Assuming, however, a rate of development proportionate with that of known egg-depositing elasmobranchs I infer that an embryo of five inches can hardly be younger than nine months (Fig. 7). An estimate of a total incubation of twelve months in this species would hardly be excessive.

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[1] A row is made up of two sets of interlocking elements, one passing to the right, one to the left, like fingers of interlocked hands.

Fertilization.—Fertilization is shark-like. Polyspermy occurs and a similar number of merocyte nuclei; the conjugation of the pronuclei occurs at a similar niveau in the germinal area, the male pronucleus passing through the germinal protoplasm and then approaching the female pronucleus from a lower plane. Also similar are the location and behavior of the merocyte nuclei during early cleavages. Different from shark, however, is the longer duration of the period of the entrance of the sperm (a newly-entered spermatozoön being present in a preparation showing fusion of pronuclei), and the clearness with which sperm paths are to be noted. Of the latter the surface pits can be seen under low powers. The germinal area is also notably deeper than in the shark.

Segmentation.—As in *Torpedo* (Rückert) and certain other elasmobranchs the appearance of cleavages at the surface of the germinal area is retarded, the first surface furrow appearing about the time of the third or fourth nuclear division. In connection with the furrows at their deeper rim are vacuoles (as in Fig. 8 at *A*) which pass into the yolk, and suggest a former condition of deeper cleavage. This inference that the fissure-like vacuoles are to be interpreted as potential but semi-suppressed cleavage spaces is well borne out by the structures of a later stage (Fig. 9) in which the vacuoles are shown to be patently continuous with intercellular spaces, and in which nuclei occur in the underlying germinal masses. It is also to be inferred that a further degree

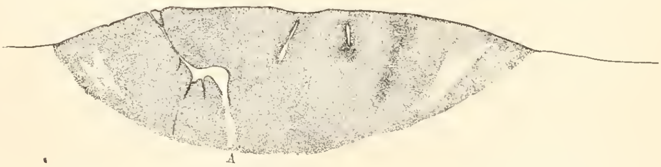

FIG. 8. Early segmentation. Section through middle of germinal area. At *A* a fissure-like vacuole becomes continuous with an intercellular space. × about 30.

in the suppression of these vacuolar cleavage spaces would result in a homogeneous and nucleated subgerminal zone. Indeed, in the present material the vacuoles do disappear from the subgerminal zone (Figs. 10 and 11), although they appear as before at the side of the germinal area, and below it, separating masses of the yolk. Note also in this connection the presence

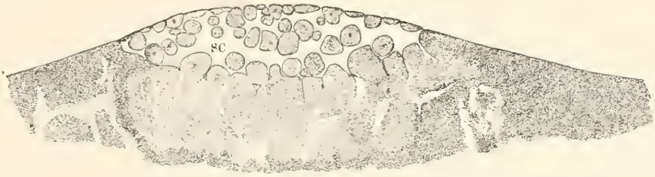

FIG. 9. Early blastula. Vacuoles are shown, continuous in arrangement with cleavage spaces. *SC.* Early segmentation cavity.

of nuclei in the marginal yolk masses thus separated as below the marks * and *, two points in the section, Fig. 11. And in another stage, Fig. 10,[1] it will be seen how far peripherad these nuclei can be traced, *e. g.*, at the points I., II., III., IV. and V. — as far peripherad, in other words, as this region of the egg has been sectioned. It follows accordingly that the yolk mass is separated from the germinal region by no means as abruptly in *Chimæra* as in the allied sharks. For in *Chimæra*,

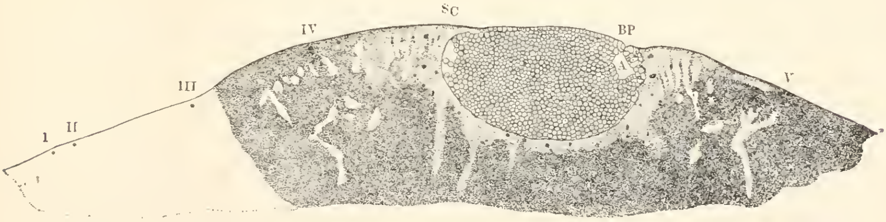

FIG. 10. Early gastrula. Sagittal section *A.* Archenteron. *BP.* Blastopore. *SC.* Segmentation cavity. I–V. Nuclei lying outside of the germinal area.

as we have seen, vacuoles which in some cases at least represent intercellular spaces pass deep into the yolk region, in the form of more or less vertical fissures. Moreover, again unlike sharks, the yolk nuclei which surround the margin of the germinal area do not occur throughout a narrow zone but extend peripherally over a wide area of the yolk. In connection with this remarkable vacuolization of the yolk mass the extension of the nucleus-bearing zone is to be considered *a process of actual fragmentation*

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[1] On one side of the present section nuclei I. and II. occur in the eighth section, and III. and IV. in the seventh and sixth sections respectively ; on the opposite side nucleus V. occurs in the fourth section.

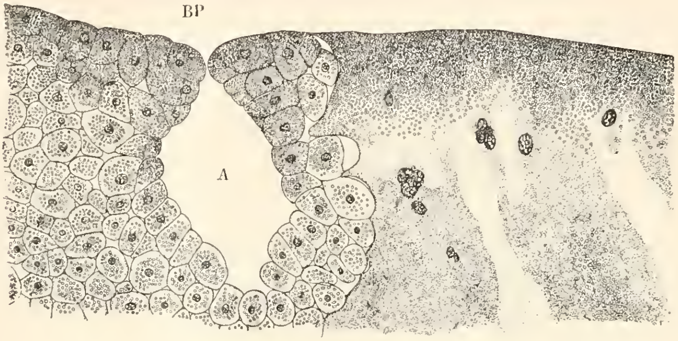


FIG. 10 A. Detail of archenteric region of preceding section.

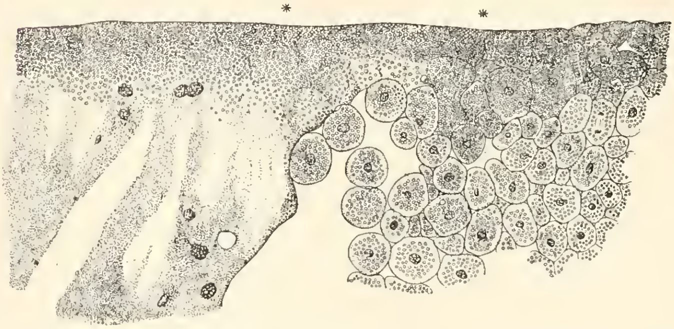


FIG. 10 B. Detail of antero-dorsal germinal wall of preceding section. Observe the cells arising in the overhanging germinal wall below and between the points *—*.

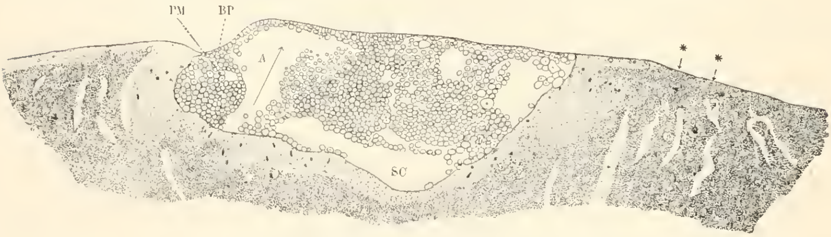


FIG. 11. Early gastrula. Sagittal section. *A.* Archenteron. *BP.* Region of blastopore. *PM.* Posterior cell mass (= ventral lip of blastopore). *SC.* Segmentation cavity. Yolk nuclei are below the asterisks. The arrow denotes the general axis of the archenteron.

of a large part of the entire egg, a process unique among vertebrates, and suggesting only remotely a physiological parallelism with developmental features in certain lower forms, *e. g.*, digenetic trematodes. Thus in a stage of late blastula the surface view of the egg (Fig. 13), shows with the unaided eye a distinct fissure passing near the side of the egg, between the points * and *, and into this run smaller ones.

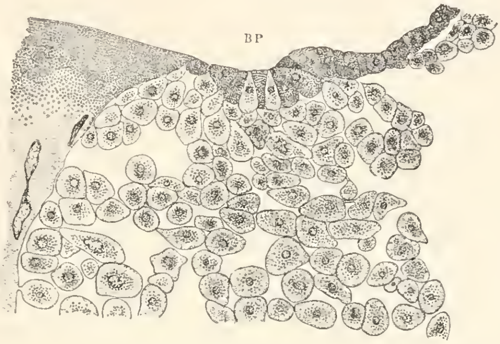


FIG. 11A. Detail of the region of the blastopore of Fig. 11.

That this is a fissure and not merely a surface marking becomes clear when the egg is hardened (*e. g.*, in corrosive-acetic), for the sides of the fissure then separate so that one can see between them deep into the yolk. In later stages (Figs. 14 and 15) similar conditions maintain, the fissures in these cases, however, extending outward from near the germinal region. In these cases it is found that the ventral side of the egg becomes more completely fragmented than the upper; thus the ventral side of the stage of Fig. 15 shows a variety of surface furrows (Fig. 16),

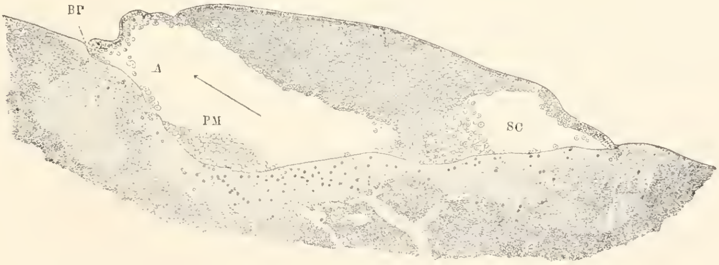
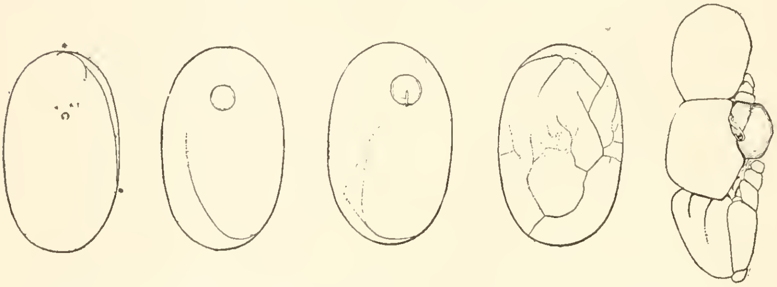


FIG. 12. Later gastrula. Lettering as in foregoing figure.

which by hardening appear as edges of deep fissures. So deeply indeed do these pass into the yolk that the writer was able by means of needles to remove a yolk mass — that lying in the middle of the lower half of the figure — almost entire. This mass, however, broke down in the process of further hardening, and no sections of it could be prepared. In later stages (Figs. 5 and 17) fragmentation continues still further. The embryo with its circumcrescent blastoderm is now attached to a relatively small portion of the yolk; the remainder is broken up into masses, big and little, and in the living egg the smallest are found dissolving into a pasty or creamy liquid, which at first sight is apt to hide the embryo and lead the observer to believe that the egg is dead and addled — an impression he soon loses when he discovers a moving embryo and finds that the fluid is odorless. In ascertaining this the writer may mention, parenthetically, that he was on the point of losing one of his most valuable stages. An egg-case was brought in which had been taken by accident, having become entangled in a trawl line; it was light in weight and its general appearance of wear and tear indicated that it was an empty shell; it was opened, however, and showed a milky fluid which suggested, by analogy of shark eggs, an unpleasantly late stage in decomposition. But as the writer began to pour out the contents of the case carelessly, he saw to his surprise a

FIG. 13 14 15 16 17



FIGS. 13–17. Eggs showing the progressive cleavage of the yolk mass. In a blastula (13) a conspicuous fissure is noted between the points * and *. In an early gastrula (14) a fissure extends hindward from near the rim of the blastoderm. In a later stage (15) a progressive fissuring of the yolk mass is seen, anteriorly and posteriorly, and notably in the ventral region (16). In the stage of Fig. 17 the entire yolk mass has undergone division, the blastoderm appropriating a single mass (slightly shaded in the figure).

mass of bright red gill-filaments. The embryo itself next appeared, as shown here in outline in Fig. 6. It was taken to the laboratory and kept living for half a day; and possibly, like kindred shark embryos, it would have thriven for weeks had one decided to rear it. Corresponding to the mass of yolk attached to the embryos in Figs. 5 and 17, the older specimen had a yolk sac of remarkably small size; the sac was complete, however, and its vitelline circulation resembled closely that of a shark. Furthermore, it may be noted that a young *Chimæra* when newly hatched has no trace of an external yolk-sac. I should record, in this connection, that my friend, Dr. Wilbur, had already (1898) observed on several occasions that the embryo with its blastoderm appropriated only a portion of the entire egg mass, and that the yolk-sac was but a miniature of a shark's. He then believed, like myself, that such a remarkable condition was abnormal. If a normal condition, however, the small yolk-sac and the fragmental yolk bear evidently upon the question of the total segmentation of this yolk-filled egg. And if the evidence is conclusive which the present observations afford, there is here given the first example of a large egg to undergo holoblastic cleavage — an interesting denial of the corollary of 'Balfour's law,' that the quantity of yolk present in an egg determines its holo- or meroblastic character. It may be well, therefore, to review in this connection the evidence of total cleavage in *Chimæra*. (1) The entire egg undergoes a progressive fragmentation, in course of which fissures first pass from the germinal region downward, and finally divide up the egg into a series of yolk masses. (2) The foregoing process is a normal one, having been observed in all specimens (later stages) examined — a dozen or thereabouts. (3) On the evidence of earlier stages distinct fissures (vacuolar) come to be formed in the sub- and circum-germinal yolk, and these are shown to be in many cases continuations of intercellular spaces of the germ itself. (4) The yolk tracts separated by (vacuolar) fissures contain nuclei; this we may conclude from the conditions pointed out in Figs. 10, 10A, and from the presence of nuclei far out beyond the germinal area (Fig. 10). In short, the egg actually undergoes total division, following a nucleation and fissuring of its yolk-substance. There is of course still the possibility that this total division is not the equivalent of total cleavage in other vertebrates, for it may be due to the action of sperm nuclei — a possibility which finds some support in the subsequent history of the extra-embryonic yolk masses (*v. infra*). This problem, however, cannot be considered specifically at the present time.[1]

Blastula. — A blastula in an early stage is drawn in section in Fig. 9. It shows a mass of blastomeres loosely arranged upon a basis of germinal cytoplasm, traversed by extensive intercellular spaces, the largest of which, *S. C.*, appears by comparison with later stages to represent the cleavage cavity. The topmost blastomeres are closely arranged and somewhat epithelial in character; the lowest are arising from the subjacent germinal well. Particularly interesting is the fissuring (vacuolar) of the subgerminal cytoplasm and of the neighboring yolk, for many of these fissure-like vacuoles are seen to be directly continuous with intercellular spaces, and are, as has above been noted, best interpreted as suppressed (or, better perhaps, retarded) lines of cleavage. Asymmetry is already present in this stage, for it will be seen that upon one side of the figure the blastomeres abut directly against the yolk, and that a more rapid increase in cells is taking place nearer the opposite side of the blastula.

Gastrula. — In Fig. 10, a section in which blastopore and archenteron appear, is represented one of the most valuable stages in my material, for I believe it furnishes us the key to the problem of the gastrulation of *Chimæra* and also probably of sharks. It shows in median section a dense mass of cells lying deep within the germinal area, and closely apposed, save at one point, (anteriormost) to the subgerminal cytoplasm. Near the opposite (posterior) rim of the cell-mass, near the surface, is a small cavity, Figs. 10 and 10 A, *A*, which communicates with the sur-

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[1] Another problem which must here be neglected deals with the important relation of yolk nuclei to mesenchyme. In this matter one may note, however, that *Chimæra* confirms Rückert's position as to the conditions in sharks. The confirmation is especially striking, since the additions to the mesenchyme budded out of the germinal wall are here of gigantic size, and cannot be confounded with the neighboring mesoblast cells.

face at the pore, *BP.* Behind it, *i. e.*, between it and the yolk, lie several rows of cells. In this cavity and its surface opening, then, we recognize archenteron and blastopore, and note further that the peculiar pigmentation of the cells of the surface of the gastrula can be followed down on either side, suggesting recent invagination, into the archenteric cavity, half way to its bottom. This, therefore, we conclude, is not a mere intercellular space with a fortuitous opening to the surface, but a definite cavity, whose cells lining the outer half are pigmented. Its cellular wall, moreover, is in general firm and compact, epithelial in character. Accordingly we must admit that in *Chimæra* a gastrula is formed whose blastopore is located not *at* the rim of the early blastoderm but *near* it. And it follows that in this condition there is still retained a phylogenetic stage in elasmobranchian gastrulation wherein the merging of the blastoderm cells into the surrounding yolk has not yet extended to that zone of the blastoderm where the blastopore is forming. It will be observed that growth is taking place at both anterior and posterior margins of the present blastoderm. Cells are coming to be budded off behind the archenteron, as well as at the blastoderm's anterior rim (Fig. 10 B) within the overlying germinal wall itself (*v.* between the points * and *). And even at this late stage blastomeres are found to be budded off abundantly from the subgerminal wall. One notes, further, that the cavity at *SC.* enlarges considerably on either side of the median line and is obviously interpreted as the segmentation cavity. In this stage many merocytes and sperm nuclei occur in the germinal wall.

In Fig. 11 is shown a somewhat later gastrula. Here the compact character of the former stage is lost, the diameter of the blastoderm having doubled. In the anterior region the segmentation cavity, *SC.*, is enlarged and is many-branched. The blastopore is obliterated, owing probably to the backward growth of the cells at the surface of the blastoderm; the archenteron, on the other hand, was greatly increased in size; its anterior wall, spreading out into a thin, somewhat epithelial layer, forms its roof, and its fundus is made up of loose cells, which focus together anteriorly. Behind the archenteron is a mass of cells, *PM.*, readily identified with that in the earlier stage. In an adjoining section, of which a detail is given in Fig. 11A, a rudiment of the blastopore of the earlier stage is shown in the region *BP.*

In a still later gastrula, Fig. 12, the prominent feature is the growth of the blastoderm hindward. The blastoderm has now increased its diameter by about one third, and its surface growth has outstripped that of the subgerminal region — in spite of the fact that the subgerminal wall has notably flattened. Thus it has come about that the posterior region of the blastoderm of the earlier stage has rolled over, as it were, the neighboring germinal wall. The point indicated at *BP.* has thus come to lie first at the edge of the blastoderm, and is next passed under its rim. And as at this point of "invagination" a separation of the cells now occurs, *this may be taken as the reopening of the blastopore.* With this rapid hindward growth the mass of cells, *PM.*, is seen to take a position apparently far forward on the subgerminal wall, but it remains in reality in its previous relative position (*i. e.*, it retains the same actual distance from the anterior border of the blastoderm). Connected again with this backward growth of the surface of the blastoderm it is also evident that the general lie of the archenteric cavity has changed, its anterior end having now been carried backward, its main length (axis of cavity) rotating somewhat as denoted by the arrows in the figures. Other symptoms of the rapid growth of the posterior rim of the blastoderm are seen in its closely compressed cells, epithelial, and in its crumpled condition. Noteworthy, further, is the solidness of the mass of cells roofing the anterior portion of the archenteron, and the reduced size and clearer contour of the segmentation cavity. It will be observed that in this stage the conditions are closely similar to those of the shark, excepting only that the archenteric and segmentation cavities have not clearly merged.

Early Embryos. — Early embryos resemble closely those of typical elasmobranchs (*cf.* Figs. 3, 4, 15); the entire extent of of their surrounding blastoderm, however, is much smaller, and the embryo attains a large size before the adjacent blastoderm surrounds a small mass of the yolk (about three eighths inch in diameter). Stages occur resembling closely sharks in Balfour's notation *C*, *D* and *E.* One might note that the head folds in the later stages are not as conspicuous. In Fig. 18 is shown a section of an embryo of about stage *C*, which is readily comparable with a corresponding stage of *Torpedo*, as figured, for example, by the Zieglers (*Arch. f. mikr. Anat.*, XXXIX., pl. IV., Fig. 19, VI.); an interesting difference is the depth to which the subblastocœlic entoderm passes into the germinal yolk at the sides of the gut, a feature which might indeed have been expected in this type of egg. In this connection observe also the fissuring (vacuolar) of the germinal yolk.

Late Embryos.—Embryos of about one eighth inch in length can be readily distinguished from those of sharks. The shape of the head is alone distinctive, for the forebrain vesicle sprouts out like a knob or crest and is a feature characteristic of embryos

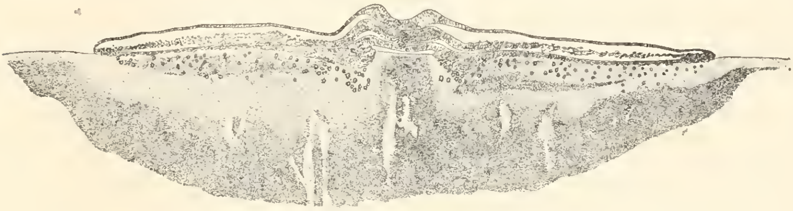

FIG. 18. Gastrula. Section through the embryonic region of the stage shown in Fig. 14.

up to about three fourths inch in length. Other characteristics are the long delicate tail and the short yolk stalk. In still later embryos external gills are developed, in a very shark-like fashion. A spiracle is present in as late a stage as one and a half inches; it is then a delicate tubular structure and is unprovided with external filaments. Peculiar to the latter is the presence of dilatations, or blood knots, about $\frac{1}{110}$ inch in diameter, brilliantly conspicuous by their scarlet color and large size in the living young. They are doubtless places of multiplication (judging from numerous mitoses) of red blood corpuscles. In this stage, as already stated, the embryo is surrounded by a milk-like fluid, which can only be derived from the continued breaking down of the separate yolk masses of an earlier stage (Figs. 5, 13–17); that it is nutritive there can be no doubt, and there is every reason for believing, judging from the physiological characters of the young of other elasmobranchs, that it is taken up by the embryo. It is probable that it is appropriated *via* gills (which may become trophonemata, as shown by Wood-Mason, Alcock, Haswell and others), and gut. This stage is of further interest from the standpoint of the morphology of the chimæroid head, for it demonstrates that the autostylous character of the skull is secondary, as had indeed been surmised from the time of Johannes Müller. As indicated in Fig. 19, from a drawing of a wax-plate model, the skull in this stage shows the upper jaw (palate-quadrate) still separate, although even at this early stage its fusion with neighboring cranial cartilage is taking place at both its anterior, and posterior borders, *A*, *B*. Conspicuous in all early stages is the disproportionate size of the anterior and posterior body regions; head and anterior trunk are large, posterior trunk and tail dwindle away narrowly, the latter, however, attaining extreme length. These characters, together with the great size of the eyes in embryonic stages, are clearly in the line of preparing the young fish for the conditions of deep-water living.

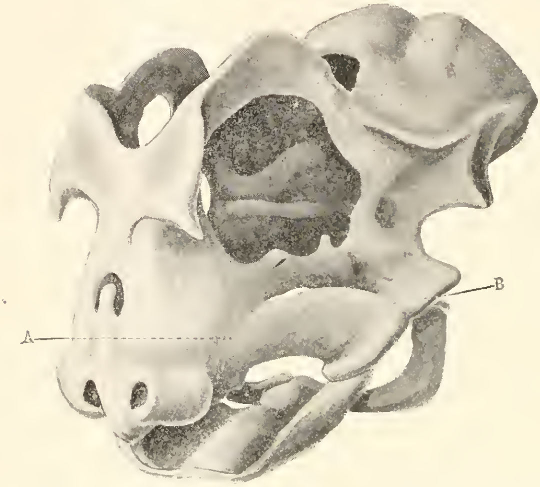

FIG. 19. Skull of embryo shown in Fig. 6. After wax-plate model. The palato-quadrate is shown fusing with the cranium at the points *A* and *B*.

Conclusions.—The mode of development of *Chimæra* affords evidence, I conclude, in support of the following theses.

I. That sharks and chimæroids are closely related genetically. Thus in comparison with other piscine groups, they are allied with one another much as earliest ganoids with the teleosts. Taxonomically we have therefore to revert to Bonaparte's early arrangement (of about 1840) and regard elasmobranchs as a subclass, and selacha and holocephala as natural orders (or superorders).

II. That in some regards, in comparison with selachians, the chimæroid has retained the more primitive developmental features, *e. g.*— the total segmentation of the egg, and the less modified early gastrula. That in other respects it has acquired more highly specialized characters, *e. g.*, restriction of the blastoderm to a smaller region of the egg, appropriation of yolk *via* the external gills (and gut), extraordinary egg capsule and its adaptation to the embryo. (The foregoing conditions, wherein high specialization is found associated with archaic developmental processes, are essentially in keeping with our knowledge of the history of the chimæroid group as derived from anatomy and palæontology. Descended from earliest sharks, this group may well have retained some of their peculiar developmental characters, *e. g.*, in earlier stages; on the other hand new and modified processes of growth doubtless arose in connection with advances which were taking place in the special direction of chimæroid structures.)

III. Of more general significance, I believe, are:

(*A*) The early processes of gastrulation in *Chimæra*, which elucidates the corresponding developmental stage in sharks. In these forms, long studied among vertebrates, gastrulation has been subject to widely different interpretations — indeed in the latest time so careful an observer as Samassa has *even denied the presence in sharks of any process of gastrulation sensu stricto. Chimæra*, it now appears, indicates that the blastopore of sharks is a secondary structure.

(*B*) The accessory mode of nutrition of the late embryo. In sharks the yolk is appropriated by means of a constant extension of the blood-producing area and a progressive differentiation of the vitelline circulation. In *Chimæra*, on the other hand, this mode of nutrition of the embryo is less extensively established, for it has been supplemented by the fragmentation of the yolk and its appropriation by external gills[1] (and gut). This process is an important one from a larger aspect since it yields a mode of nutriment hitherto unknown in vertebrate embryology — a process by which a late embryo appropriates as food, in the ordinary acceptance of the term, an outlying portion of its own organism.

(*C*) The mode of development of mesenchyme from yolk-nuclei in a somewhat similar way as described by Rückert for selachians.

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[1] The origin of the latter process is suggested on the following lines: The gills lying in close contact with the egg came to absorb nutriment from the neighboring finely divided blastomeres and from the interblastomeral fluid. And the embryo came to employ the peripheral yolk more promptly and efficaciously thus than in the ancient way, with the result that the peripheral blastomeres became more loosely associated and finally separated. These melt ultimately into a creamy fluid especially adapted for providing nutriment for the specializing gills of the embryo.

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BIOLOGICAL BULLETIN.

THE FORMATION OF THE NOTOCHORD IN THE AMPHIBIA.

HELEN DEAN KING.

A study of the mode of development of the notochord in the common toad, *Bufo lentiginosus*, and of the frog, *Rana palustris*, has brought to light certain points that have a bearing on the formation of the same structure in related groups. A vast amount of work has already been done along this line, yet a wide difference of opinion exists among embryologists regarding the origin of the notochord in the Amphibia. It is hoped that the results recorded in the present paper may help to clear up this question.

The material used was fixed in corrosive-acetic (5° glacial acetic acid), and the sections were stained on the slide with a mixture of borax-carmine and Lyon's blue as described in a previous paper (King, 11). This stain gives particularly good results when it is used on freshly preserved material, as then all of the nuclei become dark red, the ectoderm and mesoderm appear dark blue, while the yolk cells take but a pale blue tint and, therefore, are easily distinguished from the other cells. This sharp definition of the tissues was of great assistance, particularly in the study of the sections of *Bufo*. All of the drawings given in the present paper were outlined with the aid of a camera lucida.

Bufo lentiginosus.

When the circular blastopore is closing in, the mesoderm, already differentiated from the other tissues, forms a continuous sheet of small, angular, slightly pigmented cells across the dorsal wall of the archenteron. In the middle and also in the anterior part of the embryo, the mesoderm is entirely separated from the ectoderm above as well as from the endoderm beneath it. In the region just in front of the blastopore, the mesoderm is also distinct from the ectoderm, but it is united for some distance with the cells forming the dorsal wall of the archenteron. At this stage of development there is first noticed, in the middle of the embryo, a pronounced thickening of the mid-dorsal mesoderm (Fig. 1, *N*), which extends only over a few sections at first and is continuous with the lateral mesoderm on either side. When the blastopore is nearly closed, the thickened portion of the mesoderm is cut off from the lateral mesoderm to form the notochord, the line of separation coming in at about the points marked *XX* in Fig. 1. As the embryo elongates, the forward extension of the notochord always takes place in this same way, *i. e.*, by the cutting off, laterally, of a portion of the mesodermal layer in the mid-dorsal region so that, from the beginning, the notochord is entirely separated from the ectoderm and also from the endoderm. These observations confirm the statement made in a previous paper (King, 12) that "the anterior part of the notochord is certainly mesodermal in origin."

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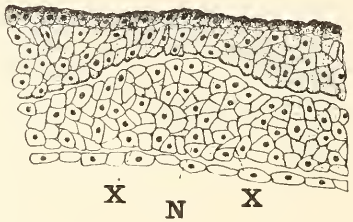

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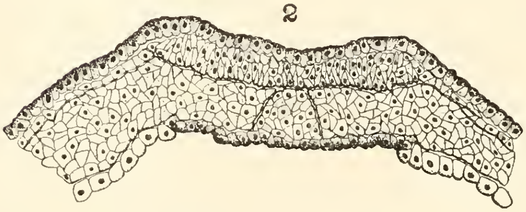
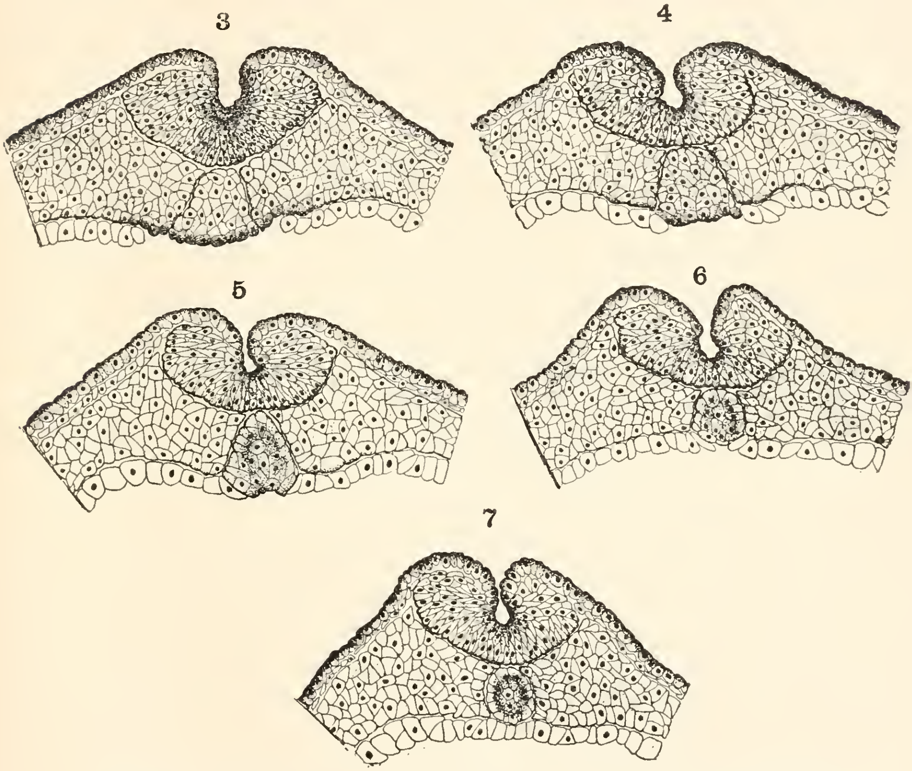


FIG. 1. Part of a medium sagittal section through an egg of *Bufo lentiginosus* in which the blastopore has begun to close. *N*, thickening of mid-dorsal mesoderm which is to be cut off at the points *XX* to form the notochord.

FIG. 2. Part of transverse section through the posterior region of an embryo in which the medullary plate has appeared.

Transverse sections through an embryo in which the medullary plate has just appeared show that, in the anterior region, the notochord is composed of a rounded mass of cells cut off entirely from the surrounding tissues, and appearing much as in Fig. 7. In the posterior region, there is, as yet, no trace of a notochord, and an unbroken layer of cells extends across the dorsal surface of the archenteron, as here the mesoderm is still united with the endoderm as in the earlier stages. In a section made a short distance behind the middle of the embryo (Fig. 2), the notochord appears as a triangular shaped chord of cells, entirely distinct from the mesodermal layer on either side, but closely connected with the cells forming the mid-dorsal wall of the archenteron. In this part of the embryo, as well as more



FIGS. 3–7. Serial sections from the posterior to the middle region of an embryo of *Bufo lentiginosus* in which the medullary folds are closing.

posteriorly, the archenteron is surrounded on its ventral, lateral and lateral-dorsal surfaces by large, rounded, faintly staining yolk cells which contain very little, if any, pigment; the mid-dorsal wall, on the contrary, is formed of a single layer of much smaller, rectangular cells which are very heavily pigmented on the side bordering the archenteron. This layer of cells, which I shall call "the dorsal plate," is broadest in the posterior part of the embryo, where, in transverse sections, it appears as a nearly straight line of cells covering about two-thirds of the mid-dorsal surface of the archenteron. More anteriorly the dorsal plate gradually becomes narrower, until it finally disappears completely in the middle of the embryo. The archenteron in front of this region is entirely surrounded by large yolk cells.

The outer cells of the dorsal plate, instead of grading into the yolk cells as one might expect, are found to be directly continuous with the lower layer of mesoderm. There is, therefore, in this region an abrupt change from the small, deeply pigmented cells of the dorsal plate to the large yolk cells which form the lateral and ventral walls of the archenteron. At no stage in the development of the embryo have I ever found any transitional stages between these two different kinds of cells. The cells of the dorsal plate resemble, in all respects, the cells forming the outer surface of the embryo, being of the same size and shape and containing about the same amount of pigment. From the results which I obtained in my study of the gastrulation of the egg of this species (King, 12), it seems highly probable that the cells composing the dorsal plate were invaginated from the surface of the egg during the formation of the blastopore, and, consequently, they have had a very different origin from the cells forming the lateral and ventral walls of the archenteron which are all derived from the yolk portion of the egg.

When the medullary folds are closing, the mesoderm in the posterior region is still connected, for a short distance, with the cells forming the dorsal wall of the archenteron, and the notochord has not yet extended into this portion of the embryo. Fig. 3 shows a portion of the section through the region where the notochord has just been cut off from the mesoderm. This section corresponds in its position in the embryo with the position of the section of the earlier embryo shown in Fig. 2. The notochord is triangular in shape and is closely connected with the layer of cells forming the mid-dorsal wall of the archenteron. The portion of the dorsal plate directly under the notochord is cut off on either side from the rest of the layer, and to it one can, perhaps, fitly apply the term "chorda-endoderm," since it is destined to become a part of the notochord. At this stage of development, the dorsal plate is much narrower in the posterior region of the embryo than it was before the medullary folds formed (Fig. 2), and it is again found to be directly connected with the lower layer of mesoderm and not with the yolk cells forming the lateral walls of the archenteron.

In Fig. 4, a portion of a section slightly anterior to that shown in Fig. 3, the chorda-endoderm is seen to be the only portion of the dorsal plate bordering the archenteron. The other cells of the dorsal plate have united with the mesoderm, and can only be distinguished from it on account of their position and the fact that they contain somewhat more pigment. The entire dorsal wall of the archenteron, excepting the part formed by the chorda-endoderm, is here composed of large, rounded yolk cells which are evidently growing up from both sides, and thus shutting off all of the cells of the dorsal plate from bordering the archenteric cavity. More anteriorly, as shown in Fig. 5, the yolk cells of the upper wall of the archenteron are still closer together in the middle lines. In this part of the embryo the cells of the chorda-endoderm no longer form a nearly straight line at the lower edge of the notochord, but they have become an integral part of it, and most of their pigment is collected in the form of a pronounced ring around the center of the notochord.

Near the middle of the embryo (Fig. 6), the yolk cells have almost met under the notochord, which is smaller and more rounded than it is in the posterior part of the embryo. A section more anteriorly still (Fig. 7) shows that the yolk cells from the two sides of the archenteron have come together in the middle line under the notochord. As a result, the dorsal wall of the archenteron is composed entirely of a single layer of large yolk cells, and the cylindrical notochord above it is cut off entirely from the surrounding tissues. In the head region, the relation of the tissues is practically the same as that shown in Fig. 7.

When the medullary folds have closed, there is found in the posterior region of the embryo a much narrower dorsal plate than that shown in Fig. 3, as more of the cells have been covered over by the upward growth of the yolk cells from the sides of the archenteric cavity. Anteriorly the dorsal plate grows narrower very rapidly and some distance back of the middle of the embryo the yolk cells have already come to surround the entire archenteron. By the time that the optic bulbs have formed, there is no longer any dorsal plate in the mid-dorsal wall of the archenteron and the notochord has no connection with any of the surrounding tissues.

These results show that the anterior part of the notochord in the embryo of *Bufo lentiginosus* is entirely mesodermal in origin; in the posterior part of the embryo, the greater part of the notochord is also derived from the mesoderm, but there is added to it a single layer of chorda-endoderm from the mid-dorsal wall of the archenteron. Back of the middle region of the embryo, the yolk cells grow up from the lateral walls of the archenteron and unite under the notochord, the cells of the dorsal plate thus cut off from bordering the archenteron, either unite with the notochord or are incorporated into the splanchnic mesoderm.

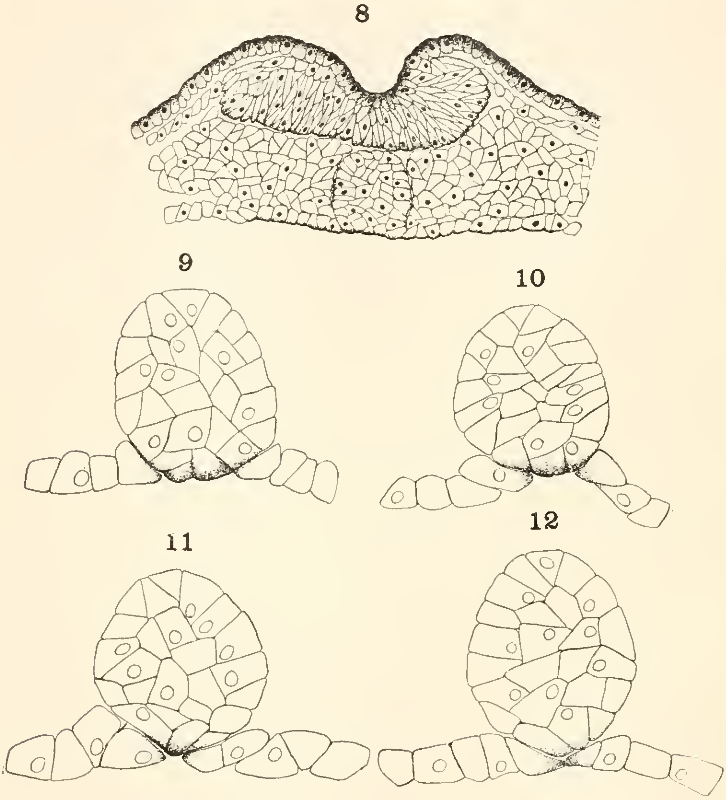
Rana palustris.

In the frog, *Rana palustris*, the notochord is formed at about the same stage of development that it is in *Bufo*, namely, near the end of gastrulation when the blastopore is closing in. As in the embryo of *Bufo*, the notochord first appears in the middle region as a rounded chord of cells cut off from the mid-dorsal mesoderm, and it is separated entirely from the ectoderm and also from the endoderm beneath which forms the dorsal wall of the archenteron. At this stage in the development of the egg, the mesoderm in front of the region where the notochord has been cut off forms a solid layer of cells extending across the dorsal wall of the archenteron and entirely separated from it; the mesoderm back of the notochord also extends in an unbroken sheet across the mid-dorsal region, but in this part of the egg mesoderm and endoderm are connected for a considerable distance on either side of the middle line.

In the posterior part of the embryo the cells forming the dorsal wall of the archenteron do not differ in size, shape, or in power of staining from the mesodermal cells above them, and at the sides of the archenteron they grade into the larger yolk cells forming the ventral and lateral walls. There is a comparatively narrow region in the mid-dorsal wall where the edges of the cells bordering the archenteric cavity are quite heavily pigmented ; but the other cells of the dorsal wall contain about the same amount of pigment as do the mesoderm cells above them, and there is no definite dorsal plate of small, deeply pigmented, rectangular cells as in the toad embryo. I can find no evidence that any of the cells of the dorsal wall of the archenteron ever form a permanent union with the mesoderm.

When the medullary folds are beginning to form, the notochord has extended into the posterior region of the embryo and appears as in Fig. 8. It is a more rounded structure than is the notochord of the toad embryo at a corresponding stage of development (Fig. 3), yet it, too, is closely connected with the endodermal layer of cells forming the mid-dorsal wall of the archenteron. As shown in Fig. 8, the lateral mesoderm and the endoderm of the archenteric wall are connected for some distance on either side of the notochord. The cells of both of these tissues have the same general characteristics, and there is no sharp distinction between them as in the embryo of *Bufo*. As all of the cells in the dorsal part of the embryo have the same power of staining, it is not easy to follow the changes that take place, even with an abundance of material for study. Not until I had made camera drawings of a complete series of sections through the posterior region of an unusually favorable embryo was I able to tell with certainty how the notochord is formed. Four of these drawings (from the same embryo as Fig. 8) are reproduced in Figs. 9–12. For the sake of clearness only the dorsal wall of the archenteron and the notochord are shown. In all of the sections the mesoderm is entirely cut off from the notochord, and also from the endoderm beneath it.

A short distance in front of the region shown in Fig. 8, almost all of the pigment in the mid-dorsal wall of the archenteron is found to be massed in the outer edges of a very few cells which are entirely cut off from the other cells of the archenteric wall and are attached to the lower surface of the notochord (Fig. 9). These few cells are undoubtedly comparable to the layer of chorda-endoderm found in the mid-dorsal wall of the archenteron in the toad embryo, and, therefore, the same term may fitly be applied to them. More anteriorly (Fig. 10) there is a noticeable upward bend in the mid-dorsal wall of the archenteron, and it appears as if the notochord with the chorda-endoderm cells is either pulling in or being pushed in from bordering the archenteric cavity, while the cells of the dorsal wall of the archenteron



Figs. 8–12. Serial sections from the posterior to the middle region of an embryo of *Rana palustris* in which the medullary folds are closing.

on either side of the notochord are coming together under the notochord. A few sections beyond (Fig. 11), the notochord is almost entirely cut off from the archenteron, as only one or two heavily pigmented cells lie between the two parts of the dorsal endoderm. In the middle region of the embryo (Fig. 12), the endodermal cells have united under the notochord and the notochord is a rounded chord of cells entirely separated from the surrounding tissues.

In *Rana palustris*, therefore, as well as in *Bufo lentiginosus*, the notochord is composed entirely of mesoderm in the anterior part of the embryo, and of mesoderm and chorda-endoderm in the posterior region. The early stages in the formation of the notochord are very similar in the two species; but in *Rana* there is no upward growth of yolk cells as in *Bufo* to form the permanent dorsal wall of the archenteron.

Most of the embryologists who have studied the early development of the Urodela agree with Jordan (10) who describes the formation of the notochord in the common newt as follows: "The cells of the median dorsal wall of the archenteron assume a somewhat columnar form and are gradually pushed up and pinched off until they are completely separated from the endoderm and come to lie above it in the mid-line." This view is held by Hertwig (7), Scott and Osborn (20), Field (5), Eycleshymer (4), Brachet (2), and Schwink (19).

Lwoff (13) is, perhaps, the most prominent of those who oppose this view. In his study of *Axolotl*, Lwoff finds that the mesoderm and the notochord are derived from cells invaginated from the surface of the egg at the blastopore rim, and he states: "Bei den Urodelen bildet sich die dorsale Wand des Darmes, ebenso wie bei Petromyzon, verhältnissmässig spät, nämlich nachdem die Chorda sich von den seitlichen Mesodermplatten gesondert hat. Die Entodermzellen wachsen von rechts und links einander entgegen, vereinigen sich unter der Chorda und bilden aufsolche Weise die dorsale Wand des Darmes." This description of the manner in which the permanent dorsal wall of the archenteron is formed in the *Axolotl* agrees remarkably well with the results of my investigations on *Bufo*. Lwoff's summary of the results of his study of the Anura based on an investigation of the early development of *Rana*, is in part as follows : " Bei den Anuren liegen insofern anderen Verhältnisse vor, also hier die Zellen, welche die dorsale Wand des Darmes bilden, von Anfang an vorhanden sind ale eine Zellenreihe und zwar als eine untere Zellenreihe jener Anlage, aus welcher die Chorda entsteht." Lwoff and I are therefore in accord in believing that in *Rana* there is no upward growth of the yolk cells from the lateral walls of the archenteron to form the mid-dorsal wall.

There is great diversity of opinion concerning the manner of the formation of the notochord in the Anura ; and, considering the careful work that has been done in this line, it seems highly probable that the process is not as uniform in this group as it is in the Urodela.

Goette (6), from his study of the development of *Bombinator igneus* concludes that in this species a central chord of mesoblast in the mid-dorsal region of the embryo separates from the two lateral sheets to form the notochord. This view is supported by the later investigations of Schultze (18), and Morgan (15) who worked on different species of *Rana*.

In a paper on the development of the middle germ layer in *Rana temporaria*, Hertwig (8) gives a number of figures of the posterior part of the embryo that bear a striking resemblance to those I have drawn of a similar region in the embryo of *Bufo lentiginosus*. Hertwig believes, however, that the entire notochord in the Anura as well as in the Urodela, is derived from a chorda-entoblast which at the sides of the archenteron pass into the endoderm cells forming the lateral walls. Field (5), from his investigations on *Rana temporaria* and on *Bufo vulgaris*, agrees with Hertwig regarding the manner of formation of the notochord, as do Robinson and Assheton (17) who worked on *Rana temporaria*. Balfour (1) also inclines to the same opinion, although he states that his evidence for so doing is not entirely conclusive.

As a result of his study of the early development of *Bombinator igneus*, Perenyi (16) advances still another theory regarding the formation of the notochord. He states that, when the blastopore closes in, " die vertikal nach innen vordringenden Zellen der Deckzellen, welche zwischen beiden Teilen des Mesoderms liegen einander berühren und sich auf der dorsalen Seite von den äussersten Zellen abzuschnüren beginnen." In this way a rod of cells is cut off from the inner layer of ectoderm to become the notochord. I know of no other investigator whose results agree with those of Perenyi.

The results which Schwink (19) has obtained from his investigations on *Rana temporaria* and *Bufo vulgaris* are very similar indeed to those which I have recorded in the present paper for *Rana palustris* and *Bufo lentiginosus*. According to Schwink, the anterior portion of the notochord in *Rana temporaria* is entirely mesodermal in origin, while the posterior part has added to it a single layer of chorda-endoderm from the dorsal wall of the archenteron, the endoderm cells at the side of the notochord growing under and uniting in the mid-dorsal line. In *Bufo vulgaris* Schwink finds that the dorsal wall of the archenteron is composed of deeply pigmented cells which, at the sides of the archenteron, pass into the larger yolk cells, although he states that in some cases it appears " dass die hier liegenden Entoblastzellen aus dem bisherigen Verband scheiden um in den Mesoblast aufgenommen zu werden." Concerning the formation of the dorsal wall of the archenteron in the posterior part of the embryo Schwink states that, " hier von beiden Seiten Darmentoblastzellen gegen die Mittellinie streben und dass dadurch Zellen, die vorher den Darm dorsal auskleideten, mit zur Bildung der Chorda verbraucht werden." This agrees exactly with what I have found to occur in the posterior region of the embryo of *Bufo lentiginosus*.

Brauer's (3) studies on the development of the Gymnophiona show that, in the posterior region of the embryo, the upper wall of the archenteron is at first formed of cells which have been invaginated from the surface. These " animal cells " are sharply marked off from the yolk or " vegetative cells " which form the side walls of the archenteron. In the anterior part of the embryo, the archenteron is extended by its connection with the segmentation cavity which is bounded entirely by yolk cells. At an early stage of development, therefore, the dorsal wall of the archenteron in the anterior region of the embryo is composed of vegetative cells, while in the posterior region it is formed of cells invaginated from the surface as I have found to be the case in the embryo of *Bufo lentiginosus*. At a later stage of development, vegetative cells grow up from the sides of the archenteron, and gradually cover up the invaginated animal cells which now form an unbroken sheet of mesoderm across the dorsal wall of the archenteron. A portion of this mesoderm in the mid-dorsal line is subsequently cut off from the lateral mesoderm to form the notochord.

In the posterior region of the embryo of *Bufo lentiginosus* a portion of the dorsal plate of cells which forms the mid-dorsal wall of the archenteron becomes cut off from the rest of the layer to be added to the notochord. If we attempt to trace the origin of this dorsal plate, we find that it is composed of cells invaginated from the surface of the egg before there was any division of the cells into ectoderm, mesoderm and endoderm. These invaginated cells form a part of the upper wall of the archenteron for a comparatively short period of development only, and those of the cells that are subsequently added to the splanchnic mesoderm soon lose their identity entirely, and cannot be distinguished in any way from the other cells of the mesoderm. The later history of the chorda-endoderm cells I have not followed.

As the endoderm cells that grow up from the sides of the archenteron and meet under the notochord are unquestionably derived from the yolk portion of the egg, the archenteron eventually becomes lined throughout its whole extent with yolk cells, and, therefore, the result is the same as if the archenteron was originally formed by a splitting between yolk cells as is believed to be the case by Robinson and Assheton (17), Houssay (9) and Moquin-Tandon (14).

According to Morgan, Wilson (21), Eycleshymer and others, there is an invagination of surface cells at the dorsal lip of the blastopore during the gastrulation of the frog's egg, and these invaginated cells come to form a part, if not all, of the dorsal wall of the archenteron in the posterior region of the embryo. In subsequent development, as the studies of Schwink and of myself show, these invaginated cells are not covered over by an upward growth of yolk cells from the lateral walls of the archenteron as is the case in the toad embryo. A few of these cells are added to the notochord, the rest, as far as I have been able to determine, remain as part of the permanent dorsal wall of the archenteron. I have never seen a section of an embryo that would warrant my stating that some of these cells become added to the mesoderm, although in the posterior region of the embryo endoderm and mesoderm are connected for a much longer time than they are in the embryo of *Bufo*.

LITERATURE.

1. **Balfour, F. M.**
'80 Comparative Embryology. Vol. II. The Macmillan Company, New York, 1880.

2. **Brachet, A.**
'02 Recherces sur l'ontogènèse des Amphibiens, Urodéles et Anoures. Archiv. de Biologie. T. XIX., 1902.

3. **Brauer, A.**
'97 Beiträge zur Kenntniss der Entwicklungsgeschichte und der Anatomie der Gymnophionen. Zool. Jahrbüch, Bd. X., 1897.

4. **Eycleshymer, A. C.**
'95 The Early Development of Amblystoma, with Observations on Some Other Vertebrates. Journ. Morph., Vol. X., 1895.

5. **Field, H. H.**
'95 Bemerkungen über die Entwicklung der Wirbelsäule bei den Amphibien; nebst Schilderung eines abnormen Wirbelsegmentes. Morph. Jahrbüch, Bd. XXII., 1895.

6. **Goette, A.**
'75 Die Entwickelungsgeschichte der Unke. Leipzig, 1875.

7. **Hertwig, O.**
'81 Die Entwicklung des mittleren Keimblattes der Wirbelthiere, I. Jena, 1881.

8. **Hertwig, O.**
'83 Die Entwicklung des mittleren Keimblattes der Wirbelthiere, II. Jena, 1883.

9. **Houssay, F.**
'90 Études d'embryologie sur les vertébrés. Archiv. de Zool. Exper. et Génér., T. VIII., 1890.

10. **Jordan, E. O.**
'93 The Habits and Development of the Newt. Journ. Morph., Vol. VIII., 1893.

11. **King, H. D.**
'01 The Maturation and Fertilization of the Egg of Bufo lentiginosus. Journ. Morph., Vol. XVII., 1901.

12. **King, H. D.**
'02 The Gastrulation of the Egg of Bufo lentiginosus. American Naturalist, Vol. XXXVI., 1902.

13. **Lwoff, B.**
'94 Die Bildung der primären Keimblätter und die Entstehung der Chorda und des Mesoderms bei den Wirbelthieren. Bull. de la Soc. Imp. des Nat. de Moscou, Bd. VIII., 1894.

14. **Moquin-Tandon, G.**

'76 Recherches sur les premières phases du développement des batraciens anoures. Ann. des Soc. Nat., T. III., 1876.

15. **Morgan, T. H.**

'97 The Development of the Frog's Egg. The Macmillan Company, New York, 1897.

16. **Perenyi, J.**

'89 Die Entwicklung der Keimblätter und der Chorda in neuer Beleuchtung. Anat. Anz., Bd. IV., 1889.

17. **Robinson, A., and Assheton, R.**

'91 The Formation and Fate of the Primitive Streak with Observations on the Archenteron and Germinal Layers of Rana temporaria. Quart. Journ. micr. Sci., Vol. XXXII., 1891.

18. **Schultze, O.**

'88 Die Entwicklung der Keimblätter und der Chorda dorsalis von Rana fusca. Zeitschr. f. wiss. Zoöl., Bd. XXVII., 1888.

19. **Schwink, F.**

'89 Ueber die Entwicklung des mittleren Keimblättes und der Chorda dorsalis der Amphibien. Munchen, 1889.

20. **Scott, W. B., and Osborn, H. F.**

'79 On some Points in the Early Development of the Common Newt. Quart. Journ. micr. Sci., Vol. XIX., 1879.

21. **Wilson, H. V.**

'00 Formation of the Blastopore in the Frog's Egg. Anat. Anz., Bd. XVIII., 1900.

ON THE COAGULATION OF THE BLOOD OF SOME ARTHROPODS AND ON THE INFLUENCE OF PRESSURE AND TRACTION ON THE PROTOPLASM OF THE BLOOD CELLS OF ARTHROPODS.[1]

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I. The Normal Coagulation of the Blood in Some Arthropods.

For this work the blood of *Limulus polyphemus*, *Homarus Americanus*, *Platyonychus ocellatus*, and some other arthropods, was used.

Coagulation of the blood takes place in a somewhat different way in *Limulus*, lobster and spider crab.

In *Limulus* the blood coagulates as a bulky mass which in the following 24 hours contracts considerably. A second coagulation of the fluid expressed from the clot may take place, to a slight extent, forming very loose gelatinous masses around the central clot, or more rarely, the whole serum may afterward coagulate.

In the lobster the first coagulum is usually relatively small, consisting of shreds; usually however, in the course of twenty minutes to an hour, the serum remaining after the first coagulation coagulates as a solid gelatinous mass which in the next twenty-four hours retracts but little. Very rarely no coagulation of the serum remains after the first coagulation takes place.

In the spider or lady-crab, the first coagulation is similar to that of the lobster blood. No second coagulation however takes place.

A combination of the following factors comes into play in producing the clotting of blood in arthropods.

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[1] This communication is in most parts only an abstract of a fuller report which shall appear later. It is intended to continue these experiments.

1. *The Agglutination of Blood Corpuscles.*—This phenomenon may be observed uncomplicated by the other factors, *e. g.*, if we collect blood in distilled water, in solutions of gelatine or of adrenalin. Under these conditions the cytoplasm of the cells itself furnishes a sticky substance necessary for agglutination. Coagulation of the blood of arthropods is partially based on *the agglutination of the blood cells taking place spontaneously outside of the body.*

2. Just as the cells agglutinate, so the cell protoplasm having left the ruptured cell body agglutinates, and the remaining part of the cell frequently has the appearance of still intact but smaller cells. The protoplasm of cells which have been absolutely dissolved may also agglutinate and form gelatinous masses.

3. About six to ten minutes after the shedding of the blood the blood cells, which in the meantime have been mostly collected in strings, begin to move in contact with solid bodies, *e. g.*, the surface of the slide; the protoplasm of the cell becomes more and more drawn out, so that ultimately a fine network of flattened cells is formed in which for a certain time the anastomosing exoplasm can be distinguished from the granular endoplasm.

4. There also takes place the coagulation of a substance which previously was in solution. This coagulation is especially marked in the blood of the lobster, but it also takes place to a less degree in the blood of *Limulus.*

II. On Some of the Factors Determining the Second Coagulation of the Blood of the Lobster.

The conditions under which the coagulation of the substance first in solution in the blood of arthropods takes place can best be studied in the blood of the lobster. If, after the shedding of the blood, one quickly collects the first fibrin by shaking the vessel and then removing it with needles, the coagulation can be inhibited for longer or shorter periods, or even be entirely prevented. The fact that coagulation nevertheless usually takes place, although somewhat later, is partially to be explained by the fact that it is impossible to remove all shreds. These shreds mostly consist of agglutinated cells. If one examines the second gelatinous coagula formed at the bottom of the dish, even after having apparently most carefully removed the shreds, one frequently sees under the microscope a small mass of blood corpuscles which had not been removed from the blood, in the center of a small gelatinous coagulum.

In many cases, however, a second coagulation after having removed most of the shreds does not take place even if some of these shreds have remained. In the cases in which it does take place it is also possible that the cell protoplasm, derived from the solution of so many blood cells, may be sufficient to cause coagulation even if the absolutely intact blood cells themselves have been entirely removed. It may be found that by adding water to the blood during the first coagulation and afterwards filtering the diluted blood, the second coagulation can be inhibited with quite a certainty for a longer period than otherwise, or may be even entirely prevented. Dilutions of the blood therefore produce an absolute diminution in the formation of fibrin for a certain period, not only a relative one in proportion to the remaining fluid.

In the experiments made such diluted serum was afterwards distributed in small dishes, 4 or 5 cc. into each dish ; different substances were added to several of those dishes to determine the influence of these substances on coagulation.

It could be shown that the addition of about two or three pieces of shreds of fibrin derived from the first coagulation of the lobster blood had a very pronounced effect on the coagulation of the diluted serum. Without the addition of such fibrin coagulation either did not take place at all, or only after some hours or on the next day. The difference in the dishes with shreds of fibrin and those without the fibrin was very marked. The influence of these shreds derived from the first coagulation was a localized one, the clotting always beginning directly around the pieces of fibrin and from here advancing concentrically towards the periphery of the fluid.

The fibrin however produced by the first coagulation is not the only substance having such an effect ; pieces of muscle of the lobster act about equally well. In these experiments the muscle was carefully washed so that all blood was removed. The fibrin of the blood of rabbits or rats, or muscle of frogs, was without effect. The fibrin of these latter animals was used as the fibrin of the lobster, or in some instances was for two hours put into lobster serum previous to the experiment, so that substances present in the fibrin which might inhibit coagulation were removed. Lobster fibrin, treated in this way, was active; the fibrin of rats and rabbits was without effect. If, however, to the lobster serum previously treated with fibrin of rabbits, fibrin of lobster was added, the serum coagulated; therefore, no substance inhibiting coagulation had been extracted from the mammalian fibrin.

Small pieces of the pancreas-liver of the lobster entirely prevented, as was to be expected, coagulation.

After pieces of the first lobster fibrin had been put for ten minutes or longer into absolute alcohol, then washed and dried, they lost almost entirely their power to produce coagulation. A trace of the second gelatinous coagulum, however, was sometimes still formed around such pieces. Control experiments showed that alcohol passing into the serum was not the cause of this loss of efficiency.

Pieces of fibrin put into chloroform water over night and even for three days, may still be very efficient, though not quite as active as fresh fibrin. Put into chloroform water for five days they lost to a large extent their efficiency. The addition of 1 cc. of chloroform water to 4 or 5 cc. of serum, or of a few drops of pure chloroform, to the serum had no more influence on the coagulation than the addition of an equal amount of water.

Heating of the fibrin in the water-bath to 46 or 47° for 30 to 40 minutes, destroyed its efficiency to a great extent, though not completely. Heating up to 51–54° for 40 minutes, destroyed its efficiency entirely or almost so.

Heating of the serum to 46–50° during 30 minutes, usually prevented the spontaneous coagulation of the serum. If, however, one adds to such serum previously heated, fresh fibrin, the serum coagulates almost as rapidly as normal serum.

The addition of 1 cc. of glycerine to 4 cc. of serum did not have any specific inhibiting influence. Also the addition of 1 cc. of a weak solution of pure gelatine did not have any specific influence.

The addition of 1 cc. of water and 6–4 per cent. sodium chloride solution to the serum had in most cases but a slightly inhibiting effect.

In such solutions coagulation has occurred occasionally even more rapidly than with the pure serum ; an accidental admixture of a larger number of blood cells to this liquid may perhaps have been the cause. All these experiments, as well as most of the following ones, were made with the addition of shreds of fibrin obtained from the first coagulation of the lobster blood.

The addition of 1 cc. of a 1 per cent. solution of potassium cyanide or of a 16 per cent. solution of Witte's peptone, or of a saturated solution of urea, had a decidedly inhibiting effect. The peptone solution prevented the coagulation entirely ; the potassium cyanide and urea solutions frequently had only an inhibiting action for some time, after perhaps 16 hours (sometimes earlier) coagulation usually occurring. Generally only half an hour after the addition of these substances their effect became noticeable. The order in which these substances, according to their strength, acted on the serum, was the following: Potassium cyanide had the weakest and Witte's peptone the strongest action ; urea stood between these substances.

If, however, the experiments were made in this way, that the pieces of the first fibrin were in the beginning put for some time into potassium cyanide, urea or peptone solutions, then after having been washed out, put into dishes containing the blood serum, the order of the loss of efficiency of the fibrin was reversed. Pieces having been kept for one half hour to two hours in peptone solution, were almost as effective as fresh fibrin, even pieces having been kept in peptone over night were frequently still very active, although in many cases they had lost a considerable part of their efficiency. It is of importance that the peptone be well washed out for some hours, otherwise the peptone, becoming extracted and dissolved in the serum, prevents its coagulation.

Pieces having been kept for one half to two hours in a solution of urea and having been afterwards well washed, have lost the greatest part of their efficiency, and after having been kept for twelve hours have become absolutely ineffective.

A 1 per cent. solution of potassium cyanide usually destroys the efficiency of the fibrin inside of half an hour. After having been kept for two hours in such a solution the fibrin becomes opaque.

One cubic centimeter of a 2 per cent. solution of potassium oxalate, or 5 drops of a 10 per cent. solution of the same solution added to 4 cc. of serum, entirely prevents the coagulation.

One half cubic centimeter of a 2 per cent. solution of potassium oxalate inhibits the coagulation markedly.

The addition of 5 drops, or 1 cc., or 2 cc. of a 2 per cent. calcium chloride solution had, in one series of experiments, a slightly accelerating influence on the coagulation in comparison with control experiments which were made with the addition of 1 cc. of a weak sodium chloride solution. Calcium is probably present in a sufficient quantity in the serum and the farther addition of it can no longer exert a pronounced influence.

A more extended series of experiments on the action of different salts on the coagulation is to be made later. Some experiments have, however, already been made on the action of ammonium chloride (the marked action of urea suggested these experiments). This salt, compared with the corresponding solutions of sodium chloride, has a specifically inhibiting action on the coagulation. The addition of 1 cc. of a 16 per cent. solution of sodium chloride frequently had a weak, though at times a somewhat stronger, inhibiting influence ; 7 drops of a 16 per cent. ammonium chloride solution, or 1 cc. of a 4 per cent. solution of the same solution, had a much more pronounced inhibiting influence. One cubic centimeter of a 16 per cent. solution of acetamid had but a slightly inhibiting effect. The addition of a few drops of ammonium prevented coagulation entirely ; this, however, was probably merely an alkali-action.

If the mixtures of blood serum with the above substances are kept for 24 hours in a warm room, putrefaction of the liquid usually takes place. This putrefaction does not prevent coagulation although it may inhibit it somewhat.

After the second, gelatinous, coagulation has taken place, it is possible to separate a second serum from this coagulum. This second serum, to which fresh shreds of fibrin have been added, no longer coagulates.

If, instead of the shreds of the first coagulation, the gelatinous coagulum of the second is added to the ordinary serum, efficiency of the latter fibrin is usually found not to be as pronounced as that of the first fibrin.

Foreign bodies, as such, added to the serum in place of fibrin or muscle, do not tend to accelerate coagulation. In no case could any marked influence be observed. Also platinum black was without any effect.

On the surface of the blood serum, kept in open dishes, usually a film is formed. This surface film in most cases radiates from the shreds of fibrin put into the serum. If we shake the serum for one half hour in dishes with uneven surfaces, macroscopically, visible membranes of fibrin are formed.

Extract of fibrin, which was previously kept in absolute alcohol, or of fresh fibrin under addition of chloroform water, have so far not shown an efficiency comparable to that obtained by the addition of fresh fibrin itself. One cubic centimeter of such an extract added to 4 cc. of serum, in most cases only caused a very insignificant acceleration of the coagulation in comparison to serum to which 1 cc. of a 6 per cent. sodium chloride solution had been added. Halliburton states that an extract of mammalian blood precipitated in alcohol and afterwards dried, produced coagulation in blood which had been collected in a magnesium sulphate solution. I did not succeed in producing coagulation in this way ; such a result would be contradictory to the one obtained by myself with the fibrin of rabbits and rats.

We may explain all these facts by the supposition that in the shreds produced by the first coagulation there is present a ferment or pro-ferment producing coagulation, and further, that such a ferment or pro-ferment must be present inside of the blood cells, because the first clot consists mainly of blood corpuscles and parts of these cells. Further, we are certain of the decidedly localized action of this ferment, as directly around the shreds of fibrin the second gelatinous fibrin is deposited. This can be explained by the assumption that the ferment can only slowly diffuse into the fluid ; it must however nevertheless be able to diffuse through the gelatinous substance formed around the shreds, although as recent experiments seem to prove, even soap mixed with gelatine is unable to diffuse through the gelatine.

The local apposition of the gelatinous mass around the fibrin shreds produces a certain resemblance between the process of clotting and that of crystallization when caused by a crystal in a solution. If we make use of the hypothesis of the action of a ferment, we have to suppose, according to the results obtained by heating the serum, that also in the serum a small quantity of this ferment or pro-ferment is present; further, that the ferment is partially destroyed by heating it up to 46–47° C. and that it is almost entirely destroyed by heating it up to 50–51° C. There is a quantitative relation present, the coagulation taking place the more rapidly the more fibrin is added to the serum. Although absolute alcohol is used to obtain mammalian fibrin ferment, and also, according to Halliburton, that of arthropods, in these experiments absolute alcohol destroyed efficiency of arthropod fibrin ferment in a very short time.

We saw that different kinds of cells and cell products of the same species of animals had the same accelerating effect on coagulation, that however the corresponding cells of other animals were without effect. The lack of specificity in the one case combined with the specificity with regard to the species of animals used, is similar to the specificity and lack of specificity respectively found in the precipitin reactions obtained after the injection of albuminous substances in different animals.

Delezenne found that contact with the muscle causes a coagulation of the blood of birds. If the contact with muscle is avoided, it is possible to keep the blood of birds liquid for a long time, although the white blood corpuscles are still present. Lobster blood, however, in contact with the blood corpuscles, does coagulate just as quickly as if in contact with muscle. These observations, notwithstanding the presence of some minor differences, seem to point out that in these phenomena we have to deal with facts of a general significance.

III. The Conditions Under Which the First Coagulation Can be Inhibited or Entirely Prevented.

The conditions under which the first coagulation can be prevented are different from those described above for the second coagulation. The following experiments were mainly made with adult Limuli :

(*a*) If one collects blood at once into saturated solutions of different salts, surpassing in quantity considerably the quantity of blood received, coagulation can be to a great extent inhibited or prevented. Magnesium sulphate, sodium chloride, potassium chloride, sodium nitrate, aluminium, ammonium sulphate, potassium oxalate, sodium citrate, potassium and sodium tartrate, potassium sulphate and sodium sulphate, all have a decidedly inhibiting effect, and to a less degree sodium carbonate.

If the solutions are diluted with one half or an equal amount of water, the effect shows a corresponding decrease. Potassium salts are less effective than sodium salts ; the potassium nitrate is almost without effect ; the oxalates do not have any markedly stronger effect than the other saturated salts ; it is therefore improbable that oxalates act in this case as precipitants of calcium, but rather as saturated salts. The blood corpuscles are in the main better preserved in these solutions. In the sodium and potassium sulphates they become roundish and send out processes after some time. In sodium nitrate and potassium and sodium chloride they retain on the whole an oval shape. Sabattani's hypothesis, that the inhibiting effect of salts is based on their action on calcium, preventing it from being ionized, becomes improbable, at least in this case. If the saturated solutions of the salts containing the blood are afterwards diluted with distilled water, many cell granules which had previously been preserved, now disappear. Many cells, however, seem to remain unchanged.

(*b*) Pyrrhol, resorcin and especially hydrochinon, have some inhibiting action on coagulation without preventing it entirely. Blood corpuscles remain more or less preserved in these solutions.

(*c*) Solutions of adrenalin chloride, 1–300, pilocarpine 1–300, and atropine 1–300, have a slightly inhibiting effect on coagulation, especially adrenalin. The cells here swell up and frequently arrange themselves through agglutination in epithelial-like order. Many cells, mainly at the margins where the adrenalin comes in contact with the blood, remain isolated or form only small clumps. The formation of a continuous coagulum however, at the margin does not take place frequently.

(*d*) A 3 per cent. solution of formalin injected into lobsters or spider-crabs has, if the blood is shed soon afterwards, a more or less inhibiting effect on the coagulation, especially if the blood is then also collected in a 3 per cent. formalin solution.

Under such conditions it can be observed that all blood corpuscles of the lobster blood have granules, and that cells corresponding to lymphocytes are normally not present in the blood of the lobster. Their presence has lately been maintained.[1]

(*e*) The first coagulation of the blood can be prevented by previously heating the animals ; the heat necessary to produce this is different in different species. The blood of small Limuli heated for 30–40 minutes up to 50–54° C. does not usually coagulate after having been shed. Lobster or *Libinia*, heated to 45–48° C. for 30 minutes loses its coagulability. Correspondingly, lobsters and spider-crabs die at a lower temperature than small Limuli. The cells usually leave the body after the animal has been heated, in a round shape and later do not usually send out pseudopodia, or do so to a less degree only. The spreading out of blood corpuscles which usually takes place under normal conditions does not take place after previous heating of the animals, or if the animal has been injected with formalin.

(*f*) Blood collected in the liver-pancreas juice does not coagulate to the same extent as ordinary blood. A large number of the blood corpuscles are usually rapidly changed into granules and many of them soon become entirely dissolved through the action of the proteolytic ferment.

(*g*) By previous injections of *Limulus* blood into turtles I have not succeeded in obtaining either precipitins for *Limulus* blood or substances which inhibit the activity of its fibrin ferment.

(*h*) *Limulus* blood collected in gelatine solutions of different concentration did not coagulate as readily as the blood collected in water, gelatine having a decidedly inhibiting action. If the solution of gelatine was very weak care had to be taken to avoid the shaking of the blood, otherwise coagulation took place. The blood corpuscles in gelatine were swollen and frequently formed epithelial-like rows.

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[1] A fuller description of the blood corpuscles of *Limulus* is given in a previous paper : *Journal of Medical Research*, Vol. II., January, 1902.

(*i*) Collected in glycerine or in chloroform the blood coagulates.

(*j*) The blood of the lobster collected in saturated urea solutions or in a 25 per cent. peptone solution, forms a gelatinous mass which does not contract in the usual way. Urea solutions have a strongly dissolving influence on the blood corpuscles. The shreds resulting from the first coagulation of the lobster blood put into urea solutions, become transparent and swell ; in glycerine they also become transparent but do not swell ; returned into water they regain their usual characters.

IV. On the Influence of Mechanical Agencies on the Protoplasm of the Blood Cells of Some Arthropods.

It has been observed by previous investigators that the blood cells may send out long processes which can adhere to solid particles. It is however possible to produce a much greater change. The protoplasm of a little clump of blood cells can be transformed into a system of threads of different sizes. It is best to use for such experiments cells which have been well preserved and are somewhat swollen ; it is also necessary to use blood in which it is possible to exclude the presence of an extra-cellular mass of fibrin. Such cells are found, *e. g.*, in blood collected in gelatine solutions or in adrenalin or, also, in hydrochinon.

The necessary traction can be applied either by putting a second slide on the first containing the blood in solution and separating the two slides after they have been pressed together or by whipping with a needle the blood cells on the slide. The whole process can be followed under the microscope ; one can see how cells arranged in a row become transformed into a system of threads which no longer indicates that it is derived from cells. Sometimes however we can see in the center of such threads the nuclei of the cells, adapting themselves to the thickness of the thread, or they may bulge out of the center of the fiber as little spheres. Under such circumstances one single cell can be changed into a very long thread. It can also be seen that frequently spindle-shaped cells arrange themselves into a single row and may form one long continuous thread passing over a large part of the slide.

Sometimes very little effort is needed to achieve such a transformation. If we move the solution containing a few drops of blood in one direction the flow of the liquid may be sufficient to arrange the cells into rows and draw them out into continuous, long threads.

Such fibrils are, however, not only formed from the protoplasm of the entire cell but they also may be formed inside of cells. This can be observed, for instance, if we exert traction by a needle in a network of cells spread out on the slide, *e. g.*, about half an hour after the blood has been shed. If we merely exert pressure on masses of cells we may change them into a structureless gelatinous mass. By exerting afterwards traction on such a gelatinous mass, using two slides, we can change this mass into a system of fibrils of different diameters; such fibrils can again be rolled up into a gelatinous mass. The protoplasm has in this case the consistency of a dough, differing however in that it is somewhat more elastic.

If we apply these mechanical agencies to cells immediately after they have left the body, we find the protoplasm more brittle and the cells can under these conditions by pressure easily be separated into small granular particles.

A transformation into fibrils can also be produced from the protoplasm which had previously left the body of the ruptured cell, or from the protoplasm of exploded cells. The same agencies, traction and pressure, are also able to make the granules of the cells disappear. Cells which had been previously kept for a longer period in saturated salt solutions or in formalin, no longer yielded such a fibrillar mass.

The physical properties of this fibrin-like mass are very similar to the fibrin formed through the coagulation of the extra-cellular part of the blood. The cellular fibrin can be drawn out, and as it is somewhat elastic, may afterwards slowly retract. It is also sticky. The cells themselves have similar physical properties. If they are spread out, as is usually the case, one half to one hour after coagulation they are kept under great tension, and if liberated from the surface of the slide by a needle they retract.

They also are sticky. A cell, hanging on a fiber after coagulation of the blood has taken place, sticks to the surface of the slide ; after having sent out processes, if as a result of outside motion a pseudopodium of the cell touches the surface of the glass.

Very similar fibrils can be produced if we subject the blood serum of *Limulus* or lobster to the same mechanical agencies. It usually however needs more exertion to obtain the same result from these colloidal solutions than from the cells.

V.

Certain analogies may be pointed out which exist between certain processes during coagulation of the blood and certain other phenomena.

(*a*) Inside of the body blood corpuscles have an oval shape and do not send out pseudopodia. After having left the body certain changes in the environment take place, the blood cells send out pseudopodia, and somewhat later they begin to spread out over the whole surface of the slide, forming one continuous network. We see a certain analogy between this process and the emigration of leucocytes under so-called inflammatory conditions.

The changes which lead to the spreading out of the blood corpuscles of arthropods cannot, under the given conditions, be determined by chemotropic influences acting from certain directions. The chemical conditions on the slide are the same on all sides ; nevertheless, the cells move and spread out. Inflammatory conditions in the higher animals also mean a change of the normal environment brought by toxic or other causes. Thus it may be that the leucocytes of vertebrates do not migrate primarily under the influence of chemotactically acting substances, but under the influence of certain physico-chemical factors which bring about changes in the state of the protoplasm of these cells ; direct chemical stimulants may be added secondarily.

(*b*) During regenerative processes of the epithelium movements of the cells take place independent of preceding cell multiplication. The epithelial cells under these conditions usually move in contact with solid bodies, parts of which, as I have previously shown, may be taken into the cell body itself. It can be observed under these conditions that the protoplasm of the epithelium is in very close connection with the solid body, for instance the coagulum forming a scab, and if one raises the scab from the wound parts of the epithelium may remain in contact with it and are raised with it. It is not unlikely that similar changes in the consistency of the protoplasm of the epithelial cells take place during regeneration, as take place in the blood cells after they have left the body, and the adhesion may in both cases have to be explained by a change in the consistency of the protoplasm which makes the cell or part of it sticky.

(*c*) We have been able to show that under the influence of traction the protoplasm of the cells assumes a fibrillar structure and that these fibrils frequently are continuous through a number of cells. Similar phenomena can be observed in many other cells. Epithelial fibrils, for instance, are not limited to one cell and especially under the influence of traction exerted during the regenerative process, the fibrils passing through more than one cell are drawn in the direction of the traction exerted and indicate therefore the movements of the epithelium. In a similar way we find fibrils passing through many cells, for instance in the heart muscle, in the developing white fibrous tissue and neuroglia and probably also in the peripheral nerves and in ganglia cells. The same mechanical factor bringing about fibrillation which can be observed experimentally in the protoplasm of blood cells, may be of importance also in these cases. The main factor is of course the specific structure of the different cells determining the specific structure of these fibrils.

(*d*) The blood corpuscles which in the body under normal conditions do not change their oval form, produce outside the body an agglomeration of cells which may be compared to a tissue. Under certain conditions, *e. g.*, in gelatine or in adrenalin, or even in distilled water, many cells form through agglutination an epithelial-like tissue ; under ordinary conditions, however the cells soon form intercellular substances and the appearance of connective tissue is produced. The network of spread-out cells closely resembles the reticular tissue. The exoplasm of the spread-out cells under the influence of slight mechanical agitation, takes on a fibrillar character. If we make sections through a coagulum in which the blood corpuscles have spread out, the appearance of connective tissue is presented. We see apparently intact cells surrounded by fibrils. These cells, however, only represent the endoplasmodic part of the original blood corpuscles, the exoplasmatic part having been transformed into fibrils. Many of these fibrils stain very well by Mallory's connective tissue stain.

In a similar way, the fibers of fibrous tissue and of neuroglia in mammalians seem to be produced from the exoplasm of the cells. In coagulating blood, however, whole cells may be changed into fibers. Here protoplasm having left the cell body and being now entirely intercellular may also form fibrils. Such an occurrence can easily be observed under the microscope in the blood, these changes taking place in a short time. It would be more difficult to prove such an occurrence in the development of connective tissue. The morphological similarity (not identity) between the processes in the blood cells and connective-tissue cells being apparently so great, it ought to be considered, whether an intercellular origin of fibers from protoplasm, secondarily disconnected from cells may not also take place in connective tissue.

We can observe that the granules of the blood cells frequently spread out and assume in the end an intercellular situation, and that they not infrequently become included in fibrils. Similar observations have been made in the developing cartilage for instance.

As we have seen, the formation of intercellular substances in the coagulating blood can be prevented if we collect the blood cells in certain solutions, which inhibit that further changes in the cells take place without preventing the possibility of such changes, if the solution is changed afterwards. We may therefore say that one of the conditions under which the formation of an intercellular substance takes place is the presence of a certain fluid which surrounds the blood cells which have left the body. The serum has therefore a more or less cytolytic power and the formation of "connective tissue" is based on a partial destruction or dissolution (cytolysis) of the blood cells by which protoplasm leaving the cells becomes changed into intercellular fibrils.

SUMMARY.

1. The following factors play a part in the coagulation of the blood of arthropods :

(*a*) Agglutination of blood cells.

(*b*) The formation of a gelatinous mass, and secondarily the formation of fibrils from protoplasm exuded from cells and from protoplasm of cells entirely dissolved.

(*c*) The spreading out of the blood cells during the next few hours after shedding of the blood.

(*d*) The coagulation of a fibrinogenous substance.

2. The agglomeration of the blood cells leads to the formation of tissue-like structures. The formation of the fibrillar intercellular substance corresponds to the formation of fibrillar connective tissue. By changing the cytolytic medium in which the cells are suspended, the formation of an intercellular fibrillar substance can be prevented. By agglutination of cells which remain well preserved, an epithelial arrangement of the blood cells can be brought about.

3. Inside of the normal body the blood cells do not change their form ; this however takes place outside of the body, without the presence of certain specifically directing chemotropic stimuli. The cell protoplasm moves in contact with the solid surface to which it sticks. In a similar way the leucocytes react during an inflammatory process and a certain analogy also exists between this process and the movement of the epithelium during regeneration.

4. Coagulation of the blood can be prevented or inhibited by receiving it in different solutions, such solutions usually having also a preservative influence upon the cells. Oxalates act in a similar way to other salt solutions, that is if they are very concentrated. This points to the fact that in all probability the precipitation of calcium is, under these conditions, not the essential factor. Sodium and potassium sulphate have, in a similar way as sodium nitrate and sodium and potassium chloride, an inhibiting influence upon coagulation. The blood cells themselves, however, are influenced in a different way by the two sulphates and the other salts.

5. The coagulation of the blood is inhibited by receiving the blood in gelatine solutions ; collecting it in oil is without influence.

6. The second coagulation of the lobster blood is inhibited by means different from those acting on the first coagulation. The

facts observed during the second coagulation can be explained through the hypothesis of the action of a fibrin ferment present in the blood cells and in the muscle of the lobster, which however is not present in the blood cells or in the muscle of certain vertebrates. This points to a multiplicity of fibrin ferments in different species of animals and points to the identity of the ferment in different tissues of the same species of animals. This specificity in the one case and the want of it in the other case corresponds to a similar specificity and want of specificity as has been found to exist with the precipitins obtained through injection of certain body fluids or albuminous substances into different species of animals.

7. Potassium cyanide, urea and solutions of peptone inhibit the second coagulation. If these substances are add to the serum their inhibiting influence is in a reverse proportion to thire strength if they act directly upon the fibrin which contains the ferment. Potassium cyanide has the strongest, peptone the weakest action upon the fibrin, and conversely, peptone has the strongest and potassium cyanide the weakest action on being mixed with serum.

8. The necessity of the presence of calcium for the second coagulation can be easily proved. Ammonium chloride has a stronger inhibiting action on the second coagulation than sodium chloride.

9. Mechanical agencies, namely, pressure and traction, change the cell protoplasm into a system of fibers which cannot be distinguished in its physical properties and appearances from the extra-cellular fibers. The cell granules disappear under the influence of traction and pressure, just as the granules disappear spontaneously in the cells during coagulation of the blood. By traction and pressure exerted upon the blood serum free from cells, similar fibrillar structures can be produced as in the protoplasm of the cell. The fibrillar structure of the protoplasm seems therefore to be a secondary condition, produced by mechanical influence upon albuminous substances which may be common to the protoplasm of the cell and to solutions of certain albuminous substances.

10. By pressure and traction the cell protoplasm can be changed into fibers whose arrangement is determined by the

direction in which the mechanical forces act ; mechanical factors may also determine the direction of the fibrils in connective tissue and bone.

11. The significance of the blood corpuscles for the coagulation of the blood consists therefore in the following : (*i*) The cells themselves are transformed into substances similar to fibrin, (*ii*) They accelerate a coagulation taking place in the surrounding fluid ; some facts point to the possibility that the coagulating substance contained in the serum has its origin in the blood cells.

12. Towards foreign substances introduced into the body of the animal, the blood corpuscles of *Limulus* do not behave as actively as the blood corpuscles of mammals. An active penetration into these foreign substances cannot be observed in *Limulus*, the cells only take part in the formation of the coagulum around the foreign substance.

PHOTOTAXIS IN VOLVOX.

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The reactions of *Volvox* to light have been studied in some detail by Oltmanns[1] who came to the conclusion that the phototactic movements of this organism are determined through the effort to reach a region of a certain optimum intensity of illumination. The theory at which Sachs and Strasburger had arrived from their studies of the phototactic reactions of plants, and which Loeb had applied to the phototaxis of animals, namely, that the direction of the movement is determined by the direction of the rays of light is not accepted by Oltmanns, but the experiments upon which this writer bases his confutation of this theory do not, I believe, bear out his interpretation. Oltmanns studied the reactions of *Volvox* to light of varying intensity by keeping specimens in a vessel so illuminated that the intensity of light gradually increased from one end to the other. This was accomplished by covering the vessel with a box the top and ends of which were made of wood while the sides were formed of narrow, wedge-shaped, hollow glass prisms filled with a mixture of India-ink and gelatine. The small ends of the prisms allowed most of the light to pass through, while the thick ends absorbed most of the rays. The light entering the enclosed vessel was obviously greatest opposite the thin ends of the prisms and gradually diminished toward the opposite end. The *Volvox* were found to assemble in different places in the vessel according to the intensity of light which fell upon the lateral prisms. With a comparatively dim outside light they would gather in the brightest end of the vessel; in light of very strong intensity they would seek the darkest end; while with moderate illumination they would take up some intermediate position. These results were held by Oltmanns to indicate that the *Volvo*. seek the places of optimum intensity of illumination and remain there, much as higher organisms collect in situations where it is neither too warm nor too cold. In the experiments of Oltmanns we certainly cannot assume that the factor of the direction of the rays has been excluded. Owing to the scattering of the rays entering perpendicularly through the sides the light end of the vessel, in such an apparatus as Oltmanns employed, must act practically as an independent source of light. If, therefore, *Volvox* is positively phototactic in weak light and negatively so in strong, we may readily understand why collections are formed in regions of a certain intensity of illumination in accordance with the theory that the direction of movements is determined by the direction of the rays.

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[1] Flora, 1892, p. 183.

As an opportunity presented itself this last fall of procuring *Volvox* easily and in large numbers I endeavored to work out some points in the phototaxis of this organism a little more in detail, and, if orientation to the direction of the rays should be found to occur, as seemed probable from the statement of previous observers, to ascertain the method by which the orienting response is brought about. It is easy to determine that *Volvox* orients itself, and that very accurately, to the direction of the rays of light. If specimens of *Volvox* are taken into a dark room and exposed to the light from an arc lamp they travel towards the light in almost a straight course, swerving remarkably little to the one side or the other. They will often travel a foot without deviating as much as a quarter of an inch from a perfectly straight course. If the position of the light is changed during their progress they soon re-orient themselves and travel straight onwards as before. If the light is placed at the other end of the vessel they turn about and come back to where they started. The shape of the *Volvox* is not quite spherical but slightly elongated, forming a prolate spheroid, and when swimming through the water the organism rotates on its long axis. As is well known the anterior end of *Volvox* may be distinguished by the fact that it is usually free from daughter colonies, and Ryder has pointed out that the red ocelli of the zoöids are much larger at this end than elsewhere and diminish gradually in size towards the posterior end of the body. While swimming towards the light the largest ocelli are always directed towards the region of greatest illumination.

That *Volvox* is negatively phototactic in strong light may be determined by exposing it to direct sunlight, or to a beam from a projection lantern after eliminating the heat rays by means of an alum cell. In negative phototaxis the body is also definitely oriented, but with the anterior end away from the light, and the organism swims away in a very nearly straight course. When it reaches a place where the light becomes less than the optimum it stops and remains comparatively quiet, only moving about slowly at intervals in an irregular manner. In very weak light *Volvox* exhibits no pronounced phototactic movements, but either lies quiet or rolls about sluggisly in various directions. With stronger illumination it becomes more active and swims straight towards the source of light, while in light of high intensity the direction of the response is reversed. We may readily understand, therefore, why in the experiments of Oltmanns the *Volvox* formed groups in regions of a certain intensity of illumination. Collections would be formed if the *Volvox* moved about irregularly without regard to the direction of the rays and came to rest when they reached a region of a particular intensity of light; but it is clear that this is not the method pursued. There are few organisms in which the orientation to the direction of the rays is more precise, or which travel to or from the light in more nearly a straight line.

How is the orientation of *Volvox* effected? It is practically impossible to determine this by studying the movements of the flagella of the individual cells, as any one who has attempted to observe these movements will easily realize. We are safe in saying that when *Volvox* changes its direction it is because the flagella on the two sides of the organism beat unequally. Can we explain the orientation, then, as a result of the fact that the differences of intensity of light on the two sides of the body cause the flagella to beat with unequal vigor so that the organism is swung around into a position of equal bilateral stimulation? It is in this way that Holt and Lee[1] have attempted to explain the orientation of *Volvox*, but there are certain difficulties in the way of such an interpretation. Let us consider a *Volvox* in a region of suboptimal stimulation and lying obliquely to the rays of light. If it orients itself to the light the backward stroke of the flagella, *i. e.* the stroke that is effective in propelling the body forward must be more effective on the shaded side than on the brighter side. This may conceivably occur in the following ways, which, however, amount practically to the same thing: The diminished intensity of light on the shaded side of the body may act as a stimulus to the backward phase, or decrease the efficiency of the forward phase of the stroke of the flagella; or the light on the brighter side of the body may inhibit the backward phase, or increase the forward phase of the stroke of the flagella. In any case, if the organism is passing into regions of ever-increasing intensity of light, we should expect its rate of speed would be lowered. If the orientation is effected by a shading of the side away from the light it would follow that in a region in which the shading were less the speed of the travelling body would be diminished. If the parts of the body which are most shaded are the parts where the effective beat of the flagella is the strongest, then, as the organism passes to a point where the illumination on both sides of its body is increased, its rate of transit would be diminished. If we suppose that the forward stroke is most stimulated, or the backward stroke most inhibited on the brightest side of the body we should expect that with more illumination the more inhibition there would be, or the more the backward phase of the stroke would be increased, and the rate of locomotion would likewise be reduced. If we imagine a machine in the form of a *Volvox* colony and provided on all sides with small movable paddles so adjusted that when they came into regions of diminished light as the machine rolled through the water their effective beat would be increased, it is clear that such a machine might orient itself to the direction of the rays and travel towards the source of illumination, but its rate of locomotion would be diminished the brighter the light into which it passed. We may conceive the light to increase or decrease the backward or forward stroke of the paddles in any way we please and we cannot explain how such a machine can orient itself and go towards the light and at the same time move through the water more rapidly as it comes into regions of greater illumination.

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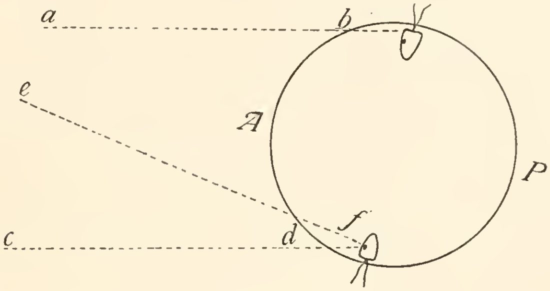
[1] *American Journal of Physiology*, Vol. IV.

Does *Volvox* react to light as the theory above mentioned would lead us to expect? Oltmanns' observation on the relation of the rate of movement of *Volvox* to the intensity of illumination, while they apparently do not conform to this theory, were not made sufficiently in detail to form a crucial test of its validity. In order to obtain evidence which would be somewhat more conclusive I placed specimens of *Volvox* in a narrow glass trough through one end of which light from an arc lamp was passed after having filtered through an alum cell. The trough was placed over a paper ruled off in spaces a centimeter in width. The specimens were placed at such a distance as was found by previous trials was about the point where they would begin to orient themselves and travel towards the light. The number of seconds required by a specimen to travel across successive intervals in its passage towards the light was noted. The experiment was repeated many times, both by using different specimens and by using the same one over again. It was found that, as the *Volvox* travelled towards the light, their movement was at first slow, their orientation not precise, and their course crooked. Gradually their path became straighter, the orientation to the light rays more exact and their speed more rapid. After travelling over a few spaces, however, their speed became remarkably uniform until the end of the trough was reached where they would remain. If the light is so intense that one end of the trough is above the optimum intensity of illumination the speed of the *Volvox* is decreased as it approaches this optimum where it finally stops. In going away from very intense light *Volvox* moves at a nearly uniform rate until within a few centimeters of the optimum when the speed begins to diminish. There is thus a lessening of speed as the optimum is approached from either direction. The distance over which there is either a marked increase or decrease of speed is considerably less, however, than the space over which the speed is nearly uniform.

When we attempt to explain the foregoing facts on the theory that orientation is effected through the differences of the intensity of light on the two sides of the organism we inevitably get into difficulties. If the *Volvox* acts as a lens concentrating the rays on the side farthest from the light so that that side is more intensely illuminated the behavior of the organism would meet the requirements of the theory. Orientation, however, occurs, apparently equally well, in those individuals which contain so many daughter colonies that a large proportion of the light is intercepted in passing through the organism; this explanation must, therefore, be dismissed even if it be otherwise valid. How then is the orientation of *Volvox* brought about? This problem is one rather more difficult to solve than it might seem. There is a suggestive similarity between the phototaxis of *Volvox* and the reactions of this organism to the electric current. Carlgren has found that *Volvox* orients itself very precisely to the constant current and swims in very nearly a straight path to the kathode. After a prolonged action of the current a more or less pronounced tendency to go towards the anode asserts itself, but the latter form of electrotaxis is much less precise and characteristic. It seems not altogether improbable that light in passing through a nearly transparent organism like *Volvox* exercises a directive effect upon its movements in a similar way, whatever it may be, to that produced by the current of electricity. The direction of the rays may be the important factor in orientation irrespective of differences of intensity of light upon different parts of the organism as has been maintained by Sachs for the phototropic movements of plants. I am not ready to adopt the theory of Sachs, but I feel that it is a view that is not entirely out of court.

There is one feature of the structure of *Volvox* which may be of some significance in relation to the problem of orientation. As Overton has pointed out, the red eye spots of the cells are so placed that they all face the anterior end of the colony. When this end is directed toward the light the spots are in a position to receive more than the usual stimulus. Orientation of the colony would be produced if each cell were to react in such a manner as to cause the eye spot to face the light. We have little direct evidence, aside from a single experiment of Englemann on *Euglena*, that the so-called eye spots of the Flagellata are photorecipient organs, but there are certain facts regarding the occurrence of these spots as well as their arrangement in *Volvox* which render it probable that such is their function. We may conceive that each cell of the colony tends to orient itself at a different angle to the rays of light, the cells of the anterior end where the largest eye spots occur placing themselves with their long axes parallel with the rays, and the other cells at various angles depending upon their position in the colony. To account for the orientation of the colony we are thrown back upon the problem of the mechanism of the process whereby each individual cell places itself so that its eye spot faces the source of light. The behavior of the cells of the colony, according to this interpretation would fall into Prof. Mark Baldwin's somewhat extensive category of imitative activity, in that each cell reacts so as to secure more of the stimulus affecting a specialized portion of its structure. How and why the cells so react we still have to explain, and various theories of orientation may be applied to the individual cells. The orientation of the colony may be accounted for rather more simply, however, if we suppose that the eye spots are most sensitive to light striking them at a certain angle such as is indicated in the diagram by the lines *ab* and *cf*. If rays of light enter the colony in the direction of the lines *ab* and *cf* somewhat obliquely to the long axis, *AP*, the flagella of the cell represented on the upper side of the diagram would beat more vigorously and accelerate the motion of that side of the organism. The opposite cell being struck by rays in the direction *cd* would be less stimulated, and, as the flagella would beat less strongly than those on the other side of the colony, the organism would swing about until its long axis is brought parallel with the rays when, being equally stimulated on both sides, it would move in a straight course towards the light. We do not have to suppose that each cell makes a special effort to orient itself at a particular angle to the rays, but that it is so organized that the effective beat of its flagella is most accelerated by light striking the cell at a certain angle. If the cells were most stimulated by light



falling upon them at such an angle as would result if the rays diverged from a spot in front of the colony and in line with its long axis the conditions for orientation would be fulfilled. Since the eye spots in all the cells face the anterior end of the colony this supposition appears very probable. The foregoing explanation of the orientation of *Volvox* may or may not be the true one, but it enables us to see a significance in the peculiar arrangement of the eye spots in this form and is consistent with the results of the experiments we have described.

How are we to explain the fact that *Volvox* becomes negatively phototactic in light of strong intensity? We certainly cannot explain the reversal on the supposition that it is due to a difference of emphasis in the phases of the stroke of the flagella. In positive phototaxis the backward phase of the stroke of the flagella is the stronger, and if we suppose that with increase of stimulation the reverse phase comes to predominate the organism would go backwards instead of forwards. As it is the anterior end of the organism that is directed away from the light in negative phototaxis it is obvious that the same phase of the stroke of the flagella predominates is both cases. The theory of Holt and Lee applies here very well; if the effective beat of the flagella is greatest on the more illuminated side the organism would naturally turn about so as to point away from the light. This may or may not be the true explanation of the negative reaction; I see at present no way either of proving or of disproving it. The probability of the supposition is somewhat weakened, however, by the fact that it cannot also be applied to positive phototaxis. It seems probable that light accelerates the action of the flagella not only by stimulating the eye spots but by acting on other parts of the cells, and we might suppose that, as the light is increased, a point would be reached where the stimulus to the other parts of the cell would outweigh the effect upon the eye spots, and the flagella on the side of the organism nearest the light, beating more vigorously than the others, would thereby bring about the negative orientation. We can thus give at least a formal explanation of the phenomenon. There are many instances in which an increase in the intensity of a stimulus causes a reversal of the usual reaction, but the reason for this in any case is still an unsolved problem.

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